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BLINDNESS AND OTHER DISEASES IN CHILDREN ARISING IN CONSEQUENCE OF DEFICIENT NUTRITION (LACK OF FAT SOLUBLE A FACTOR)[1]

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While blindness in children has been on the decrease in the greater part of the civilized world, the opposite has taken place in Denmark. Blindness in children has increased not only absolutely but also relatively to the population. Many causes have contributed to the diminution of blindness in other countries, but the chief one undoubtedly is that gonorrhoeal opthalmoblenorrhoea in the newborn has become a rare disease as a result of the special measures which have been adopted against it everywhere. That gonorrhoeal opthalmia has become a rarity also applies to Denmark but in spite of this the percentage of blindness in children is increasing.

There is however, another disease of the eye peculiar to childhood which leads to blindness. This is xerophthalmia. It was first described in the middle of last century by German ophthalmologists. According to all previous reports xerophthalmia had a very serious prognosis as it occurred particularly in the final stage of a cachectic condition due to a protracted wasting disease such as tuberculosis, syphilis, typhoid fever, acute or chronic digestive diseases, etc., and especially in badly nourished and debilitated children.

In the middle and close of last century and in the beginning of this, "epidemics" of an eye disease were described which were undoubtedly indentical with xerophthalmia. These "epidemics" occurred among negro children in Brazil, during periods of

[1] This paper was read by Dr. C. E. Bloch before the World's Dairy Congress, Washington, D. C., October 3, 1923.

fasting in Russia, when famine and summer diarrhoea accompanied one another in Japan, and it was always the badly nourished and diseased children of the poorer classes who were attacked. The majority of the authors who have described these epidemics therefore associate xerophthalmia in children with a bad and deficient diet. Primitive medicine in Japan, Russia and other countries has from ancient times treated this eye disease with fat substances, especially cod liver oil and liver, and Mori has declared that the disease is due to lack of fat in the diet.

In Europe xerophthalmia is considered to be extremely rare. A few cases have previously been recorded in badly nourished children in England and Denmark, while in Germany, Czerny and Keller and, according to them, several others, have now and again observed xerophthalmia in the final stage of the nutritional disease they call "Mehlnahrschaden."

The Children's Department of the Danish State Hospital was opened in 1911. Even by the next spring a number of children were admitted with a curious eye disease which proved to be xerophthalmia, and in the following year the number increased to an alarming extent. I thus, had the opportunity of studying this disease in its different forms and stages, about which I published communications, some long and others short, in 1914, 1916, 1917, 1918 and 1922.

My investigations indicated that the disease was due to a lack of a particular substance. This substance was present in fresh milk, but not in separated or butter milk. It was therefore united with the butter fat and it was also found in abundant quantities in cod liver oil. My investigations further showed that the disease could not be attributed to the absence of fat as such, because even though the children had received margarine or pork fat they could easily get xerophthalmia.

In England and America (U. S. A.) a large number of experimental investigations into nutrition were carried out partly before and partly at the same time as my work. From these enquiries we became further acquainted with the cause of xerophthalmia and the disease associated with it. It was from the animal experiments of Osborne and Mendel, and especially of

McCollum and his collaborators that we know now the cause of xerophthalmia is the lack of the fat-soluble A factor. This body which is united with certain fat substances and is notably found in large quantities in butter and cod liver oil, must be constantly supplied to the organism, particularly the growing organism, in order that growth shall take place; deprivation for a long period leads to xerophthalmia and death.

When a child, particularly one in the first few years of life, is deprived of its mother's milk or fresh milk, as well as cream and butter, and is kept for a long time on a diet consisting of puddings made with separated or butter milk, bread and other farinaceous foods, potato-mash, a little pork and other meat, margarine, and pork fat, it will become ill as a result of the absence of the fat-soluble A factor. Even if the child eats a little vegetable, fruit and green-stuff now and again it will not help much, for a small child can only digest trifling amounts of green-stuff.

A deficiency of the fat-soluble A factor may also occur in children who have received a quantity of pure milk but in such cases the milk must have been stale and modified adversely, the fat-soluble A factor being destroyed by oxidation and repeated heating.

The disease is ushered in by the child ceasing to gain weight. Later on there is a loss of weight and the child remains motionless and apathetic. In other words there is *inhibition of growth* and depressed *psychic and bodily vitality*.

These children are liable to be attacked by some infectious disease which usually takes the form of a simple catarrhal infection of the mucous membranes particularly of the respiratory organs and middle ear. *B. coli* infections of the urinary tract and infectious dermatitis are also frequently observed. These infections are extraordinarily persistent and often have a vital issue. An *increased susceptibility* and a *diminished power of resistance* against infections are therefore present.

The eye lesion does not appear as a rule until late in the disease. It first occurs in the form of night-blindness

(hemoralopia) and dryness of the ocular conjunctiva (xerosis). The dryness later extends over the cornea which becomes hazy, shrivels up, and shortly after, pieces of it become necrossed (keratomalacia). In most cases the ocular conjunctiva is infected; the child then has photophobia, the conjunctiva is red, swollen, and the eye begins to water. When the cornea necroses the inflammation spreads to the interior of the eye. The in-

Fig. 1.

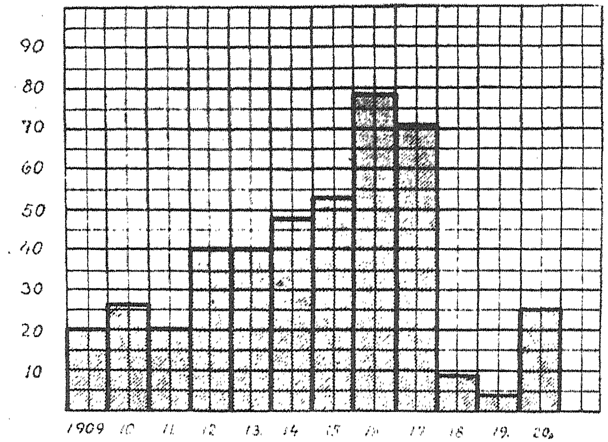

FIG. 1. CHART SHOWING NUMBER OF CASES OF XEROPHTHALMIA FOR 1909 TO 1920 IN DENMARK

Note the remarkable drop in the year 1918 and 1919 when the government rationed 0.25 kgm. of butter a week to each person in the kingdom. In 1920 rationing ceased; note increase in disease.

flammation may be so intense and widespread in many cases that it almost obscures the original xerosis. The eye lesion can only be distinguished with difficulty from the destructive inflammation in such cases.

If the disease is not recognized in time the child becomes blind. If the child does not then die of a secondary infection first, the most frequent cause of death is pneumonia.

In figure 2 such a patient is seen, a boy of about two years. He appears to be a healthy child, eyes only being swollen and

kept tightly closed. He is however, of small size and weighs less than normal. He also has a catarrhal infection. His eyes held

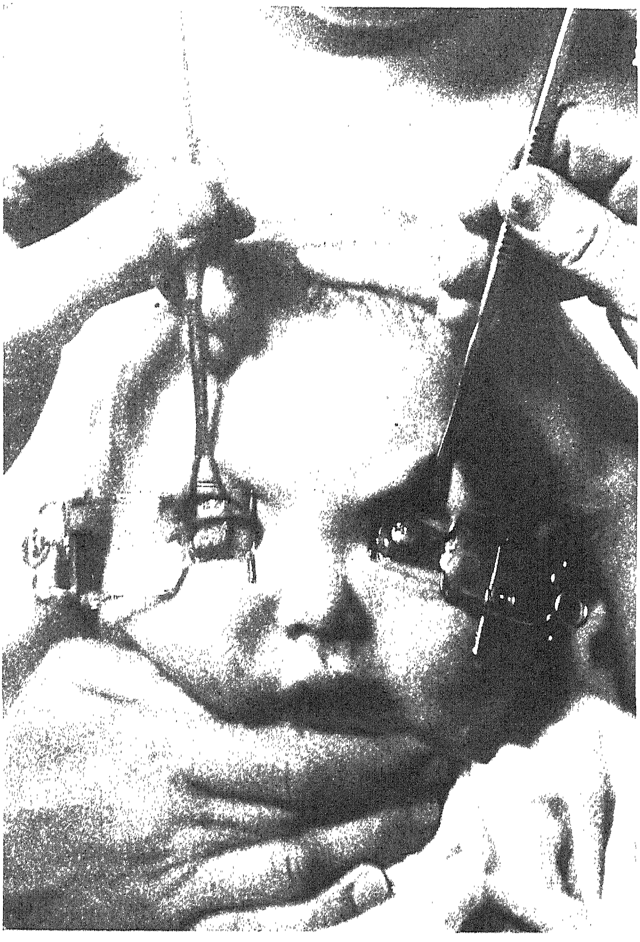

FIG. 2. SHOWS EYE DISEASE KNOWN AS XEROPHTHALMIA, MORE OR LESS COMMON AMONG CHILDREN FED A DIET WITHOUT SUFFICIENT BUTTERFAT

open are seen to be completely destroyed. The conjunctiva is swollen and red, both corneae are necrotic and there is pus in the anterior chamber.

The child was cured but became totally blind as the cornea never heals with clear transparent tissue. The scars are always hazy and opaque. In cases where the cornea is only partly damaged the child however may usually be saved from complete blindness.

If a small child receives no milk whatever, not even separated milk or butter milk, but is only given water, tea, gruel and the like for a prolonged period on account of diarrhoea or other

FIG. 3. SHOWS HOW XEROPHTHALMIA IS ARRESTED WHEN BUTTERFAT IS ADDED TO RATION

malady, the child will diminish in size and become atrophic, with dry wrinkled skin, but otherwise it will resemble the first type.

In one such case, in addition to the absence of the fat-soluble A factor several others are missing, but it is essentially the primary wasting disease—in this instance the intestinal trouble which dominates the entire clinical picture. Such a case answers most nearly to the xerophthalmia described by the older authors.

When the child is a little older and stronger, and not ill, it can apparently thrive for a long time on infant food or other essen-

tially carbohydrate diet, but sooner or later, the tissues become oedematous, and if the child has received no fresh milk, xerophthalmia and pneumonia, etc., may supervene just as in the first type.

If the child had only a little butter in addition to the carbohydrate diet, oedema, like that in Bright's disease, can certainly occur but neither xerophthalmia nor marked changes in resisting power are observed, and the child is easily restored to health when it received milk.

Besides the xerophthalmia the characteristic symptoms in this disease which arises in consequence of the absence of the fat-soluble A factor are therefore inhibition of growth and diminished immunity and vitality. In bigger children and adults who hardly utilize so much fat-soluble A factor as the small child, the disease may be merely represented by the latter symptoms. It is only when the lack of the fat-soluble A factor in the food has been almost absolute for a long time or when the consumption of it has been very great, e.g., in chronic infectious diseases, that the bigger children get xerophthalmia also. It is chiefly the small children, that is to say those under two to three years of age, that get xerophthalmia and this may happen even though there has been some fat-soluble A factor in their food.

During the war there was much sickness among the bigger children in central Europe, which took the form especially of inhibition of growth, and a large number of infectious diseases running a grave course. Thus many children were attacked by tuberculosis. The smaller children on the other hand were remarkably little affected and xeropthalmia was hardly seen at all in them. This state of affairs was due to the fact that during the war all the milk and milk products in Germany were rationed so that every little child obtained a certain amount daily. If the child was being suckled by the mother she got the milk and xerophthalmia was never observed in children suckled by a mother capable of yielding sufficient milk.

In Denmark the conditions, as mentioned, were different; before and especially during the war there were many cases of xerophthalmia. In a recent investigation undertaken for the

Danish Ophthalmological Society 600 to 700 cases of xerophthalmia were found to have occurred between the years 1909 to 1920 in children in a population of less than three millions, and in addition to these there were probably others.

The reason xerophthalmia was so widespread, despite the fact that Denmark did not take part in the war, is that this country is a dairy-farming one which manufactures and exports butter. During the war butter, and consequently fresh milk, became so dear that the poor could scarcely procure any of it and they were only able to obtain separated and butter milk which could always be had at a very low price. The number of cases of xerophthalmia increased therefore until 1918. In that year the disease was suddenly checked as will be seen from the chart. The reasons were several but the principal cause was undoubtedly that in 1918, on account of the German blockade, butter was rationed in Denmark, so that every individual including small children was entitled to 0.25 kgm. butter a week, and as there was practically no pork fat or margarine in the country everybody had to eat butter. Xerophthalmia ceased simultaneously. These facts show as clearly as an experiment that the absence of the fat-soluble A factor is the cause of xerophthalmia.

After the termination of the war the rationing of butter came to an end and xerophthalmia returned but it was rare compared with its former incidence. It was particularly noticeable that the severe cases no longer occurred. The people and the Danish doctors had got to know the disease and had learned how to avoid and treat it.

The disease caused by lack of fat-soluble A factor is cured by supplying the latter to the afflicted organism, best in the form of fresh milk, cream or cod liver oil. The xerophthalmia is the first to disappear; then the child begins to thrive and gradually throws off the infections. The latter are therefore cured by the administration of the fat-soluble A factor. This is in harmony with the old clinical experience that chronic infectious diseases, particularly tuberculosis, are improved by a diet rich in fresh milk, butter and cod liver oil.

The fat-soluble A factor is thus particularly important not only for the eye lesion but also for establishing immunity, and it is clear that a great deal of it is used up in infectious diseases. Large quantities must also be consumed during growth. This is most clearly evident from McCollum's animal experiments, but the study of xerophthalmia points in the same direction, as it occurs practically only in children, that is to say, in growing individuals. Moreover the disease appears especially in the spring months at which season the most marked growth of the child takes place. The cases occurring in this annual period of growth have always been complicated by serious infections or chronic diarrhoea.

It has been thought that the accumulation of cases in the spring was due to a different content of fat-soluble A factor in the winter milk (stall feeding) and the summer (milk grass feeding). It is possible that there is more fat-soluble A factor in the first "grass-milk" and that this has something to do with the disappearance of the disease in the summer, but a decreased fat-soluble A content in the milk cannot be the cause of the many cases arising in the growth-period of the spring because precisely the same distribution is found of cases that have received no milk or milk products at all for a long while.

This special relation between the fat-soluble A factor and growth, and between it and infections, has not been thought to be confined to this body but is also found in other deficiency diseases. Thus Alfred F. Hess has specially pointed out that the relation is somewhat similar in the case of scurvy, the disease which arises from the absence of the anti-scorbutic factor. Scurvy, like every other serious disease is undoubtedly able to affect growth and diminish the power of resistance against infections, but in the disease caused by lack of the fat-soluble A factor both these features are very strongly emphasized and in a special manner. Thus scurvy in contra-distinction to xerophthalmia is not particularly associated with childhood nor does it like xerophthalmia appear especially during the annual growth period. Moreover a statistical examination of my cases of scurvy shows

that this disease is far from being associated with infections to the same extent as xerophthalmia.

Finally, I will just mention that xerophthalmia and rickets are only very rarely met with together in the same child. Rickets can therefore hardly be due to the absence of the fat-soluble A factor in the food or in the organism.

What I have said there and proved to you, testifies to the enormous importance of milk as a food for the child. No other article can replace milk. Absence of milk from the diet or the inclusion of unfavorably modified milk is the origin of most serious diseases.

By ordering milk and especially cream and butter not only is this terrible eye diseases cured—which I believe will be discovered in every country when it is looked for—but these dairy products are of the greatest importance for growth and development, and for the cure of our greatest infectious diseases.

ON THE PROTEIN REQUIREMENT OF MILK PRODUCTION

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This experiment, conducted between February 28 and May 1, 1919, had for its object the determination of the smallest amount of a certain mixture of proteins which it is necessary to supply, in excess of the requirement for maintenance, in order to support the production of milk without drawing on the protein of the body. This study was undertaken as a war-time activity, a study of economy in the production of an indispensable food.

On account of the depletion of the staff of the Institute through military service it was impossible to prosecute this investigation on the most critical basis, and allowance for this unavoidable situation should be made. In spite of certain compromise procedures in method, however, the results are not without effect to contribute enlightening evidence.

The subjects of the experiment were 2 mature Jersey cows, nos. 869 and 884, aged four and six years, respectively, the former having calved 142 days, and the latter 58 days, prior to the beginning of the first experimental period.

With cow 869, therefore, the experiment covered days of lactation 143 to 205, and with cow 884, days of lactation 59 to 121.

At the beginning of the first collection period cow 884 had been bred 2 days, and cow 869 had been pregnant for 72 days. During the experiment, then, cow 884 was in days of gestation 3 to 65, and cow 869, days of gestation 73 to 135.

These 2 cows differed markedly in their character as milk producers, no. 869 being a persistent milker, of normal dairy disposition to produce milk, while no. 884 was not so good a producer,

and was characterized by having a short lactation period, and by a noticeable tendency to fatten. These points are of importance in relation to the behavior of these cows in the experiment to be discussed.

The general method of experimentation was by balances of intake, in the feed, compared with outgo in the milk, urine and feces, the experimental subjects being maintained in metabolism stalls so constructed as to provide for the collection of urine and feces together.

TABLE 1

Composition of feeds

FEED	DRY MATTER	TOTAL NITROGEN	FACTOR FOR PROTEIN	CRUDE PROTEIN	DRY BASIS: Crude protein	Digestibility coefficient protein	Digestibility, crude protein	Factor, true protein	Digestibility, true protein	Therms net energy, 100 pounds
	per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	
Alfalfa hay.....	91.585	2.321	6.25	14.506	15.839	72	11.404	71/106	7.639	37.451
Corn meal.....	88.393	1.362	6.00	8.172	9.245	66	6.102	64/69	5.660	96.054
Linseed oil meal.	90.246	5.692	5.50	31.306	34.690	89	30.874	285/302	29.136	97.811
Peanut meal....	90.765	5.740	5.50	31.570	34.782	90	31.304	414/428	30.280	104.759
Oat straw......	93.130	0.526	6.25	3.288	3.531	33	1.165	8/10	0.932	39.333
Corn starch....	86.637	0.256	6.25	0.160	0.185	50*	0.092	1/10*	0.009	81.790

* Assumed.

The separate amounts of urine and feces were computed from the combined weight by the use of percentage figures based on crude fiber estimations on the combined excreta and on the feces alone as separately collected by attendants. This separate collection was made from 8:00 a.m. until 4:00 p.m. on two days at the end of each experimental period, these days being the last one of the experimental period proper, and the first thereafter.

The ration was maintained, during these two days, exactly the same as during the remainder of the experimental period.

The course of the experiment was followed by daily weights of the cows, before and after drinking, weights of combined urine

and feces, determinations of nitrogen in the excreta, weights of milk, and determinations of nitrogen in the milk.

The plan covered five periods of two weeks each, the protein content of the rations varying from period to period, but the energy being maintained constant.

As planned, the protein to be fed, in excess of an arbitrarily assumed maintenance requirement, was to be computed from the amount of protein in the milk produced during the preceding period, by multiplication by the following arbitrarily determined factors:

Period I. 1.6 times the milk protein
Period II. 1.3 times the milk protein
Period III. 1.0 times the milk protein
Period IV. 1.3 times the milk protein
Period V. 1.6 times the milk protein

TABLE 2

Daily rations of cows—kilograms, fresh basis

COW NUMBER		PERIOD I	PERIOD II	PERIOD III	PERIOD IV	PERIOD V
869	Alfalfa hay................	3.696	3.130	2.606	2.899	3.696
	Grain.....................	4.790	4.060	1.361	3.765	4.790
	Oat straw................		0.908	3.375	1.802	
	Starch....................		0.460	1.047		
884	Alfalfa hay................	3.871	3.130	2.534	2.314	3.498
	Grain.....................	5.022	4.060	3.282	3.001	5.022
	Oat straw................		1.340	1.948	2.435	
	Starch....................		0.262	0.916	0.995	

Note: The grain mixture was composed of corn meal 77.09 per cent, peanut meal 11.21 per cent, and linseed oilmeal 11.70 per cent.

Cow 884 refused 3.391 kgm. feed in period I, and 0.059 kgm. in period IV.

In the discussion of the experiment, however, it will be noted that this schedule of protein allowance was not followed exactly. In certain instances it was changed voluntarily; and in all cases the production of milk differed more or less from the previously computed amount. There are, therefore, considerable differences between this adopted schedule and the corresponding ratios which actually prevailed during the course of the experiment.

It was planned that each fortnightly period be subdivided as follows:

	days
Preliminary feeding period	5
Digestion period	7
Calculation of ration for next period	2

This plan prevailed throughout the experiment, except that the 5 day preliminary period was, in 5 cases out of the 10, shortened to 2 or 3 days by the necessity for readjusting the amount of feed.

The rations were composed of alfalfa hay, corn meal, linseed oilmeal, and peanut meal, with the further addition of oat straw and corn starch in certain periods to adjust the energy value of the ration.

Each ration contained the four feeds first mentioned in such amounts to supply true protein in the following proportions:

	per cent
By alfalfa hay	35
By corn meal	25
By linseed oilmeal	20
By peanut meal	20

In the adjustment of the energy value of the rations to the uniform plane of intake contemplated by the plan of the experiment the digestible true protein content of the oat straw and of the corn starch were considered as negligible.

TABLE 3

Daily rations of cows—kilograms, dry matter

COW NUMBER		PERIOD I	PERIOD II	PERIOD III	PERIOD IV	PERIOD V
869	Alfalfa hay	3.263	2.786	2.311	2.587	3.282
	Grain	3.999	3.437	2.863	3.195	4.071
	Oat straw		0.809	1.218	1.626	
	Starch		0.395	0.902		
884	Alfalfa hay	3.418	2.786	2.247	2.065	3.106
	Grain	4.192	3.437	2.784	2.546	4.268
	Oat straw		1.194	1.743	2.198	
	Starch		0.225	0.789	0.858	

Note: Cow 884 refused feed in period I to the amount of 0.356 kgm. per day, which is equivalent to 4.7 per cent of the total. This refused feed was analysed, and accounted for, in so far as this was possible.

The feeds used were analyzed in advance, and the rations computed from these analyses, assuming average coefficients of digestibility.

The rate of feeding was computed on the following basis: Maintenance requirement of digestible true protein, 0.5 pound per 1000 pounds live weight; digestible true protein of feed, in relation to protein content of milk in preceding experimental period, allowed at computed rates varying from 96.6 to 205.4 per cent of the milk protein, plus the maintenance requirement of protein; energy supplied, in relation to energy content of milk in preceding period, 105 per cent, plus energy requirement for maintenance, in accord with the Armsby standard of net energy.

The rates at which digestible true protein was actually fed, in comparison with the milk protein of the preceding period, are the following, the numbers being those by which it would be necessary to multiply the milk protein to equal the true protein of the feed.

PERIOD	COW 869	COW 884
I	1.60	1.60
II	1.30	1.30
III	1.027	1.028
IV	1.28	0.966
V	1.869	2.054

In comparison with the milk protein of the concurrent period the true protein of the feed was as indicated by the following factors, the significance of which is the same as in the above table:

PERIOD	COW 869	COW 884
I	1.67	1.78
II	1.36	1.46
III	1.08	1.14
IV	1.32	0.95
V	1.74	2.07

During the preliminary feeding, and also during period I cow no. 884 refused varying quantities of feed each day, the amounts

during the 3-day intermediate feeding being 0.700, 0.152, and 0.450 kilo; and during the 7-day collection period, 0.250, 0.402, 0.486, 0.978, 0.680, 0.574, and 0.021 kilo.

During the 5-day preliminary feeding of period IV this cow refused, per day, 0.078 kilo of feed; and in the collection period following she refused 0.059 kilo of feed.

Since the refusal of feed introduces error of undeterminable magnitude into metabolism experiments that data relating to cow 884 in periods I and IV rest upon a less certain basis than do the other results of the experiment.

Another unfavorable condition which requires mention is the shortness of the collection periods (7 days), and especially of the preliminary feeding periods (2 to 5 days).

The length of preliminary periods, as above stated, does not in all cases cover the entire time of preliminary feeding but only that portion of this time during which the amount of feed consumed was exactly the same as that given during the collection period to follow:

Length of preliminary feeding periods

PERIOD	COW 869	COW 884
	days	*days*
I	3	3
II	2	3
III	5	5
IV	5	5
V	5	3

Neither the preliminary nor the collection periods were of length adequate for the intended purpose, that is, to obtain evidence as to the protein requirements of milk production.

It is also unquestionably true that the basis of evidence upon which the amounts of urine and feces were computed, from the weight of the two combined, as collected, was slight.

However, in spite of those unfavorable conditions the results, as set forth in the tables, bring out clearly certain points of interest and value.

Table 4 sets forth the average daily amounts of nitrogen in the feed, milk and excreta, and the balance of income to outgo of nitrogen.

TABLE 4

Daily balances of nitrogen—grams

COW NUMBER		PERIOD I	PERIOD II	PERIOD III	PERIOD IV	PERIOD V
869	Feed	203.3	173.6	149.7	167.7	207.4
	Milk	50.5	48.3	46.9	44.9	48.4
	Excreta	160.5	128.4	105.4	120.1	142.4
	Balance	−7.7	−3.1	−2.6	+2.7	+16.6
884	Feed	205.4	175.3	148.7	138.8	208.5
	Milk	49.0	43.7	40.2	40.4	40.2
	Excreta	155.8	132.7	103.1	101.0	155.2
	Balance	+0.6	−1.1	+5.4	−2.6	+13.1

Note: Excreta, in this computation, includes urine, feces and brushings (shed hair and scurf).

TABLE 5

Relation of protein intake to production and requirements—daily averages

COW NUMBER	PERIOD NUMBER	AVERAGE WEIGHT OF COW	FAT CONTENT OF MILK	AVERAGE DAILY MILK PRODUCED	DIGESTIBLE CRUDE PROTEIN: Actually fed	DIGESTIBLE CRUDE PROTEIN: Computed maintenance requirement*	DIGESTIBLE CRUDE PROTEIN: Available for production	RATIO SYNTHESIZED TO AVAILABLE NITROGEN†
		kilos	*per cent*	*kilos*	*grams*	*grams*	*grams*	
	I	370.6	5.19	8.422	799.4	222.4	577.0	1:2.16
	II	371.9	4.91	8.013	688.1	223.1	465.0	1.65
869	III	372.5	5.18	7.495	547.5	223.5	324.0	1.17
	IV	374.3	5.26	7.488	651.9	224.6	427.3	1.44
	V	370.8	5.11	8.117	895.0	222.5	672.5	1.66
	I	409.2	4.72	8.544	898.1	245.5	652.6	2.10
	II	410.6	5.14	7.565	666.3	246.4	419.9	1.58
884	III	409.0	5.43	6.957	561.9	245.4	316.5	1.11
	IV	415.9	5.42	6.853	492.5	249.5	243.0	1.03
	V	406.3	5.60	6.891	940.0	243.8	696.7	2.09

* The maintenance requirement was assumed to be 0.6 kgm. per 1000 kgm. of live weight.

† The available nitrogen is computed by subtraction of the maintenance requirement from the digestible nitrogen of the ration. The synthesized nitrogen is the nitrogen of the milk plus positive and minus negative nitrogen balances.

These figures show that the milk-producing cow adheres to her natural rate of milk production with remarkable persistence, in spite of extensive change in the nitrogen intake.

Considering, first, cow 869, as the protein intake decreases from 203.3 to 173.6 and from 173.6 to 149.7 grams per day, 53.6 grams in all, the nitrogen in the excreta decreases almost an identical amount, 55.1 grams, while the loss from the body seemed actually to decrease, though these variations may be within the range of experimental error, and therefore not significant. At the same

TABLE 6

Daily utilization of nitrogen

COW NUMBER	PERIOD	DAYS OF LACTATION	NITROGEN INTAKE	COEFFICIENT OF DIGESTIBILITY	NITROGEN IN MILK	BODY GAIN OR LOSS IN NITROGEN	TOTAL NITROGEN SYNTHESIZED	NITROGEN SYNTHESIZED	AVAILABLE NITROGEN* SYNTHESIZED
			grams	*per cent*	*grams*	*grams*	*grams*	*per cent of intake*	*per cent*
	I	142–148	203.28	62.94	50.45	−7.68	42.77	21.04	46.33
	II	156–162	173.56	63.46	48.29	−3.14	45.15	26.01	60.69
869	III	170–176	149.73	58.52	46.88	−2.55	44.33	29.61	85.51
	IV	184–190	167.69	62.23	44.93	+2.13	47.56	28.36	69.56
	V	198–204	207.35	69.05	48.40	+16.53	64.93	31.31	60.34
	I	58–64	205.41	69.96	49.04	+0.60	49.64	24.17	47.54
	II	72–78	175.30	60.81	43.92	−1.15	42.57	24.28	63.36
884	III	86–92	148.70	60.46	40.16	+5.41	45.57	30.65	89.99
	IV	100–106	138.83	56.73	40.38	−2.56	37.82	27.24	97.27
	V	114–120	208.51	72.13	40.23	+13.10	53.33	25.58	47.84

* Available nitrogen is digestible nitrogen minus the maintenance requirement, and the utilized protein is the the protein of the milk plus positive and minus negative protein balances.

time the outgo of nitrogen in the milk decreased only from 50.5 to 46.9 grams per day.

This extensive decrease of outgo of nitrogen in the excreta signifies a decreased utilization of protein for fat and energy production, and, arithmetically at least, an increased efficiency in the utilization of the decreased intake, for milk production.

This observation is further illustrated by the figures in the right-hand column of table 6. These data (cow 869) make it seem that the utilization of protein available for milk production

had increased from 46.33 to 85.51 per cent as the proportion of the protein intake utilized for milk production was increased through the decrease in the utilization of protein for energy and fat production.

This cow (no. 869), having calved 142 days before the beginning of period I, was in the fifth and sixth months of lactation during Periods I, II, and III.

According to Hills[1] a cow normally decreases in milk production at a rate of one-tenth of the yield of the preceding month, for the first nine months of lactation. It seems likely, therefore, that the rate of decrease in milk production during periods I, II, III, was about normal, but the fact that the percentage of the total nitrogen of the rations synthesized (utilized for body gain or milk production) increased during these periods (see table 6) implies, at least, that the lowest rate of intake was closer to the rate of greatest efficiency than was the next higher. The failure of this cow to increase in milk production with the increase of nitrogen intake in period IV, however, though the increase was appreciable in period V, exhibits such a tendency for the milk production to lag behind the changes of rations as makes it uncertain whether the minimum milk production of period IV, really represents more clearly the feed of this period or of period III. These results, especially the relative constancy of milk production, suggest the necessity of very much longer periods if the results are to be used as indications of economy of production of milk.

With cow 884 the course of results was similar, in that, with progressive decrease in protein intake, in periods I, II, III, and IV, there was associated a progressive increase in efficiency of utilization of protein. With decrease of nitrogen intake there was appreciable decrease of nitrogen outgo in the milk, the minimum being reached in period III. The minimum nitrogen intake in period IV was reflected in decreased outgo in the excreta, and increased loss from the body, but did not cause further decrease in the nitrogen outgo of the milk. The extensive increase of nitro-

[1] Vermont Agric. Exp. Sta., Bul. 225, p. 146.

gen intake in period V, was reflected in prominent increase of nitrogen outgo in the excreta, and extensive increase in nitrogen retention, but on account of the lag of the milk production behind the ration changes, there was no improvement in milk production in this period.

With both cows the percentage utilization of available nitrogen decreased prominently coincident with the increase of protein intake, in periods IV and V with cow 869, and in period V with cow 884.

It is obvious, therefore, that the rate of intake characterized by most efficient use of protein would be reached only on such reduced protein intake that no protein (or at least the minimum amount possible) would be utilized for fat or energy production.

It will be noted that these data, which we have discussed, on efficiency of utilization of protein, have been based on the available protein, that is, the digestible protein in excess of the maintenance requirement.

More significant figures, from a practical point of view, would be those representing rate of efficiency of utilization of protein, based on the total protein intake.

Such data are set forth in the second column from the right in table 6, but these particular data are of such nature, on account of the lag of changes in milk production behind changes in protein intake, that it is impossible to say which of the past levels of intake is responsible for a given result.

All things considered, however, it appears that the rates of allowance of protein which prevailed in period III, with both cows, were most satisfactory, since in these cases there was neither extensive gain or loss of protein by the body, while high rates of efficiency in the production of milk prevailed, on the basis of both the available protein and the total protein intake.

The rate of nitrogen intake in these two cases was 148.70 to 149.73 grams total nitrogen. This supplied 1.11 to 1.17 times as much available nitrogen as was synthesized, and 1.027 to 1.028 times as much digestible true protein (in excess of the maintenance requirement) as was contained in the milk during the second week previous.

The more or less definite indication is, then, that after allowing for maintenance at a rate of 0.6 pound of apparently digestible crude protein per 1000 pounds live weight, the most efficient rate of crude protein intake for milk production, under the conditions of a metabolism investigation, with milk averaging 5.13 to 5.26 per cent fat content, is approximately 1.11 to 1.17 times the amount of the protein in the milk. The evidence in favor of these particular figures, however, is not conclusive, and more extensive data will doubtless modify them.

The practical significance of these data is indicated by a comparison of the protein intake of a cow fed in accord with these results, with the protein requirement of the same cow, according to Henry and Morrison's standard.

A 1000 pound cow, producing 40 pounds per day of milk containing 5 per cent of fat and 3.5 per cent of protein, would require, according to Henry and Morrison, 3.36 pounds of digestible crude protein per day. The same cow, fed in accord with the results of this experiment and on the particular rations used, would require 34.5 per cent less protein, or 2.20 pounds.

We do not mean to imply, however, that the most efficient rate of protein intake, as determined in a metabolism experiment, will cover all exigencies of practice. Requirements of practice must be determined under conditions of practice.

Our computations happen to have been based on an assumed maintenance allowance of 0.6 pound, instead of 0.7 pound of digestible crude protein according to Henry and Morrison, but this makes little difference in the result, since the assumption of a lower maintenance figure for protein arithmetically increases the allowance of available feed protein per pound of milk protein.

The most that can be concluded from the decreasing negative balances of nitrogen coincident with decreasing intake of nitrogen in periods I, II, III, if indeed the differences in these balances are significant, is that this decreasing rate of intake had an effect to induce more efficient utilization of the nitrogen of the ration. Having attained this habit of efficiency the cow was in condition to store nitrogen liberally with the increased intake in period V. The extensive improvement of the nitrogen balance in period V,

with slight increase in the nitrogen of the milk, is concordant with the common knowledge that after a decrease in milk production, in response to unfavorable conditions of feeding, it is very difficult, at any time in the same period of lactation, to bring the cow back to the normal rate of production.

A point of interest in connection with the coefficients of digestibility is that, in 7 cases out of 8, whenever the nitrogen intake was increased the coefficient of digestibility of protein was increased, and when the nitrogen intake was decreased the digestion coefficient likewise was decreased; which signifies that, with this ration, undigested nitrogen was more nearly a constant function of the animal than of the ration.

In this connection let us consider the fact that digestible protein, as determined in this experiment, was in reality, only *apparently* digestible, and that the figures for digestible protein were low by the amount of the undetermined metabolic nitrogen of the feces.

Were the figures for available protein low by the same amount? It seems not. Apparently the metabolic protein of the feces at least approaches a constant, related more definitely to the animal than to the feed, being represented, therefore, by a part of the maintenance allowance of protein. When the apparently digestible protein was decreased by the amount of the maintenance requirement, in reckoning the available protein, it was, in effect, corrected for the metabolic nitrogen of urine and feces together, since these comprise the maintenance quota.

SUMMARY

After allowing 0.6 pound apparently digestible crude protein (0.5 pound true protein) per 1000 pounds live weight for maintenance, an intake of crude protein equal to 1.25 times the milk protein of two weeks previous (true protein, 1.03 times the milk protein) caused a slight nitrogen loss with one cow, and a slight retention of nitrogen with the other. The intake, therefore, was close to the requirement.

In the same experimental periods the total synthesized protein (milk protein plus positive or minus negative protein balances)

was 0.85 to 0.90 of the amount of the crude protein available for increase (protein intake minus the maintenance requirement).

It is recognized that this rate of efficiency of production of milk, in relation to the protein intake, applies less definitely to practice than to the conditions of these metabolism experiments.

Reduction of the protein intake from the larger to the smaller amounts fed had the effect to reduce the milk flow. Subsequent increase of protein intake caused a significant increase of milk production with the cow of more pronounced milk-giving tendency (no. 869), but no significant increase with the poorer cow (no. 884), while both cows responded to the increase of protein in the ration by considerable storage of nitrogen in the body.

With a low protein intake the utilization of protein for milk production was more efficient than with an intake of protein sufficient to permit of more extensive use for fat and energy production.

There is, with the cow, a prominent inclination to produce milk at an individual rate which is determined by inheritance. On account of the ability of the cow to deflect protein from energy production, and to withdraw protein from the tissues, and to use the same, in both cases, for milk production, the immediate effect of reduction of protein intake to reduce the production of milk is only partial in degree, the maximum effect being reached after a period which may extend to a number of weeks.

The apparent digestibility of protein by the cow increases prominently with increase in the protein intake, this effect apparently being due to metabolic protein being more nearly a body constant than a variable related directly to the feed.

THE EFFECT OF GESTATION UPON LACTATION IN THE DAIRY COW

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The nutrients required to develop the bovine fetus in the non-lactating dairy cow have been studied by Eckles (1) and Hill (2). Eckles, from a maintenance trial with 4 cows, concludes that the amount of nutrients necessary to develop the bovine fetus is so small that it cannot be measured by ordinary methods of experimentation. This, he thinks, is due to the fact that the amount of dry matter contained in the fetus and its accompanying fluid and membranes is very small,—the Jersey calf at birth being only equivalent to from 110 to 170 pounds of Jersey milk and the Holstein calf from 200 to 275 pounds of Holstein milk.

Hill, from a maintenance trial with 6 cows, found that fetal construction makes a relatively small draft upon the carbohydrate content of the ration, if one considers only the figures representing ultimate product and does not take cognizance of the draft made by the metabolic processes involved in fetal construction. The protein content of the ration, however, was somewhat more heavily drawn upon—to the extent of approximately 10 per cent of the gross digestible protein intake.

Since the publication of Eckles work with non-lactating cows, it has been quite generally inferred that gestation has no more effect upon milk production than that which may be accounted for by the dry matter content of the fetus.

The object of this paper is to report the results of a study to determine the effect of gestation upon the lactating dairy cow especially as it affects the rate of decline and total milk secreted. One method of attack was to select two groups consisting of animals from the Advanced Register of Guernsey Cattle. Included in the first group were all cows that were bred during the third and fourth months of lactation whereas only farrow

cows were selected for the second group. The average monthly milk production of the non-pregnant and pregnant cows was then determined by means of correlation tables. The results presented in figure 1 and table 1 show that during the first five months of lactation the farrow and the pregnant cows decline in

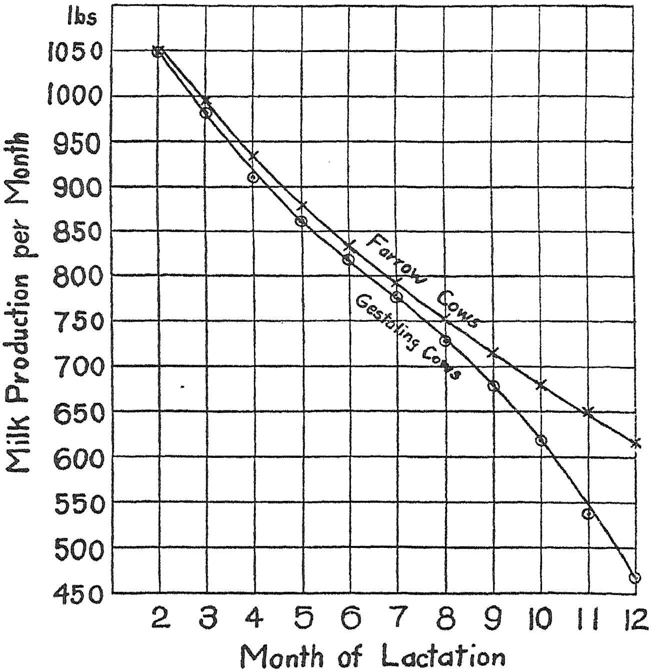

FIG. 1. THE AVERAGE MONTHLY MILK PRODUCTION OF NON-PREGNANT AND PREGNANT COWS

During the first five months of pregnancy both groups of cows decline in milk production in a similar manner. After the fifth month and until the end of the twelfth month of lactation the pregnant cows decrease in their milk flow more rapidly.

milk production in a similar manner. After the fifth month and until the end of the twelfth month of lactation the pregnant cows decrease in milk flow more rapidly. It was pointed out in a previous paper (3) that there is a law governing the decline of milk secretion with the advance of the period of lactation of cows

under favorable conditions of feeding and management. This law may be expressed as follows: Each month's production after the second month is a constant percentage of the preceding month's production (94.77 per cent in the case of the cows under consideration). This law holds true to a remarkable degree with the non-pregnant cows but in the case of the pregnant cows, there is a more rapid decline during the last four months of lactation. This indicates that pregnancy or the accompanying physiological changes going on during the latter stages of pregnancy cause a

TABLE 1

The relative decline of milk secretion with the advance of the period of lactation of farrow and gestating cows (Guernsey breed)

MONTH OF LACTATION	FARROW COWS			GESTATING COWS	
	Number of animals	Yield per month		Number of animals	Yield per month (observed)
		Observed	Calculated		
		pounds	*pounds*		*pounds*
2	920	1052	1049	373	1052
3	912	998	994	374	983
4	923	938	942	373	911
5	914	879	874	372	862
6	917	833	846	372	819
7	912	792	802	374	776
8	904	752	760	371	728
9	905	715	720	369	679
10	906	676	682	369	618
11	852	650	641	358	538
12	653	617	613	283	469

greater decrease in the rate of milk secretion than that due entirely to the advance of the period of lactation. The total decrease in the data studied amounted to over 480 pounds of milk.

A second method of study consisted in keeping the stage of lactation constant and varying the stage of gestation. This was accomplished by grouping cows for the twelfth, eleventh, tenth and eighth months of lactation according to the time after breeding. Table 2 presents the complete data. Figure 2 pic-

tures the effect of the stage of gestation for the eighth and twelfth months of lactation. This data shows a rapid decline in milk secretion after the fifth month of gestation.

The effect of pregnancy on yearly fat production is shown in table 3. It will be noted that the decline of fat production is greatest with cows pregnant two-hundred days or more.

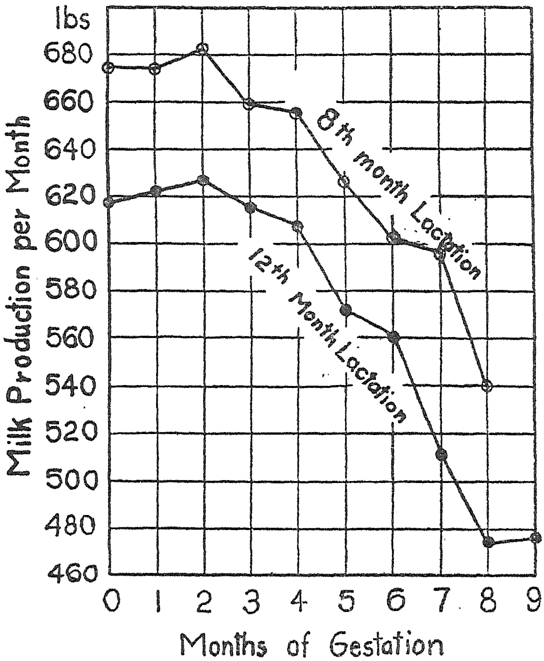

FIG. 2. THE EFFECT OF THE STAGE OF GESTATION WHEN THE PERIOD OF LACTATION IS CONSTANT

After the fifth month of gestation there is a rapid decline in milk secretion

When all age classes are combined there is a decrease of 40 pounds of fat between the cows pregnant not to exceed fifty days as compared to cows pregnant over two-hundred and fifty days. This amounts to a reduction of 800 pounds of 5 per cent milk.

The data presented indicate that gestation is the cause of a reduction of milk amounting to between 480 and 800 pounds

TABLE 2

The effect of the stage of gestation on the milk flow

MONTH OF GESTATION	EIGHTH MONTH OF LACTATION		TENTH MONTH OF LACTATION		ELEVENTH MONTH OF LACTATION		TWELFTH MONTH OF LACTATION	
	Number of cows	Milk yield per month	Number of cows	Milk yield per month	Number of cows	Milk yield per month	Number of cows	Milk yield per month
		pounds		*pounds*		*pounds*		*pounds*
0	906	752.8	907	676.2	854	651.4	654	616.8
1	887	736.4	393	673.9	315	682.9	276	621.9
2	1113	740.8	616	681.0	413	649.7	307	626.8
3	959	615.6	890	657.7	611	647.6	399	615.5
4	667	707.1	1133	655.1	869	616.0	593	626.6
5	319	707.8	975	626.9	1099	625.5	861	570.7
6	19	647.2	659	602.3	942	575.0	1085	562.2
7			294	595.9	620	539.8	909	508.4
8			18	536.6	272	524.7	563	471.7
9					14	527.1	199	474.1
10							11	561.8

TABLE 3

Effect of pregnancy on yearly fat production

(Guernsey yearly records)

DAYS PREGNANT	MATURE COWS		SENIOR FOUR-YEAR-OLDS		JUNIOR FOUR-YEAR-OLDS		SENIOR THREE-YEAR-OLDS		JUNIOR THREE-YEAR-OLDS		SENIOR TWO-YEAR-OLDS		JUNIOR TWO-YEAR-OLDS		ALL CLASSES COMBINED	
	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat
		lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*
0–50	398	544.37	71	519.02	99	503.99	96	469.23	117	438.35	127	420.65	309	403.08	1,217	474.70
50–100	325	543.89	57	516.18	57	504.90	74	505.84	91	452.80	127	456.31	237	422.99	968	487.40
100–150	502	528.87	99	515.42	102	497.12	139	481.97	149	453.18	166	425.46	433	410.59	1,590	471.79
150–200	868	522.40	166	508.42	231	531.50	265	481.77	247	448.91	360	430.79	724	405.15	2,861	471.02
200–250	764	502.11	172	472.07	218	470.76	224	449.05	286	425.18	348	413.31	693	392.45	2,705	445.63
250–300	563	488.91	144	468.65	175	450.92	189	437.78	226	414.79	227	400.71	436	374.53	1,960	434.90
300–up	15	420.68	4	454.58	4	464.58	2	332.37	9	364.43	10	360.31	11	547.42	55	386.81
Total	3,435		713		886		989		1,125		1,365		2,843		11,356	

Note: This table includes all A.R. records from 1 to 9800 inclusive, and re-entries.

during the latter part of the lactation period. In order to interpret this data, it is necessary to have in mind the physiological changes that take place in the maternal organism during pregnancy.

With the onset of pregnancy, the dairy cow must not only provide the several nutrients for the production of milk but she must also provide the protein and energy necessary for building up and maintaining the developing fetus. During the early stages of pregnancy the embryo is so small in comparison to the weight of the mother that the nutrients required are insignificant. After the fourth or fifth month, however, the fetus has developed sufficiently to require an appreciable amount of nutrients for growth and maintenance. It has been shown by Bar (4) and Hoffstrom (5) that the nitrogen requirement is greatly increased during the eighth and ninth months of pregnancy. Magnus-Levy (6) and Carpenter and Murlin (7) have also shown the increased energy metabolism of pregnancy.

The drafts for nutrients required by the developing fetus may be in part compensated for by the greater retention of nitrogen during the latter stages of pregnancy (8). The metabolism may also be lowered by decreased activity of the dam. Armsby (9) has shown that metabolism decreases 30 to 40 per cent while the animal is at rest as compared to standing.

With a pregnant non-lactating dairy cow on maintenance the lowered metabolism and greater nitrogen retention might easily provide the nutrients for fetal maintenance and growth. On the other hand the nutrients available in the blood of the pregnant lactating dairy cow during the latter months of gestation must of necessity be divided between the needs of the fetus and the mammary gland.

It is believed that the reduction in the milk flow of the lactating dairy cow gives a better picture of the nutrients required to develop the bovine fetus than does the dry matter content of the fetus expressed in pounds of milk.

TABLE 2

The effect of the stage of gestation on the milk flow

MONTH OF GESTATION	EIGHTH MONTH OF LACTATION		TENTH MONTH OF LACTATION		ELEVENTH MONTH OF LACTATION		TWELFTH MONTH OF LACTATION	
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	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat	Number of cows	Fat
		lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*
0–50	398	544.37	71	519.02	99	503.99	96	469.23	117	438.35	127	420.65	309	403.08	1,217	474.70
50–100	325	543.89	57	516.18	57	504.90	74	505.84	91	452.80	127	456.31	237	422.99	968	487.40
100–150	502	528.87	99	515.42	102	497.12	139	481.97	149	453.18	166	425.46	433	410.59	1,590	471.79
150–200	868	522.40	166	508.42	231	531.50	265	481.77	247	448.91	360	430.79	724	405.15	2,861	471.02
200–250	764	502.11	172	472.07	218	470.76	224	449.05	286	425.18	348	413.31	693	392.45	2,705	445.63
250–300	563	488.91	144	468.65	175	450.92	189	437.78	226	414.79	227	400.71	436	374.53	1,960	434.90
300–up	15	420.68	4	454.58	4	464.58	2	332.37	9	364.43	10	360.31	11	547.42	55	386.81
Total	3,435		713		886		989		1,125		1,365		2,843		11,356	

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With the onset of pregnancy, the dairy cow must not only provide the several nutrients for the production of milk but she must also provide the protein and energy necessary for building up and maintaining the developing fetus. During the early stages of pregnancy the embryo is so small in comparison to the weight of the mother that the nutrients required are insignificant. After the fourth or fifth month, however, the fetus has developed sufficiently to require an appreciable amount of nutrients for growth and maintenance. It has been shown by Bar (4) and Hoffstrom (5) that the nitrogen requirement is greatly increased during the eighth and ninth months of pregnancy. Magnus-Levy (6) and Carpenter and Murlin (7) have also shown the increased energy metabolism of pregnancy.

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With a pregnant non-lactating dairy cow on maintenance the lowered metabolism and greater nitrogen retention might easily provide the nutrients for fetal maintenance and growth. On the other hand the nutrients available in the blood of the pregnant lactating dairy cow during the latter months of gestation must of necessity be divided between the needs of the fetus and the mammary gland.

It is believed that the reduction in the milk flow of the lactating dairy cow gives a better picture of the nutrients required to develop the bovine fetus than does the dry matter content of the fetus expressed in pounds of milk.

SUMMARY

Data is presented showing that the effect of pregnancy becomes apparent in a reduced rate of milk secretion when during lactation the period of pregnancy exceeds about five months. The total reduction may amount to from 480 to 800 pounds of milk if cows are bred during the early months of lactation. The data indicates that this reduction of milk flow is caused in part at least by the demand of the fetus for nutrients to support its life processes.

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MEASURING QUALITY IN ICE CREAM

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Uniform good quality ice cream must be one of the biggest assets in the continued expansion of the ice cream industry. What are the factors that make up quality in ice cream? The butter and cheese industry solved these questions sometime ago and a uniform system of grades and standards have been developed. So far no such grades and standards have been developed in the ice cream industry. Different score cards and other systems of measuring quality have been suggested from time to time, but none of these have become standardized. In view of the fact that ice cream is now included in the Educational Students Dairy Products Judging Contest at the Eastern States Exposition, and that serious consideration is being given towards including ice cream in the National Contest, it seems very desirable to establish some uniform system of measuring the quality of ice cream. It is with this idea in mind and in the hope of opening up a thorough discussion on the subject that the writers suggest a score card and a system of grades and standards. According to this score card, the factors that make up quality in ice cream are: Flavor, body and texture; bacteria color and package. The score card measures these quality factors by assigning to each a certain value which is as follows:

	per cent
Flavor	50
Body and texture	25
Bacteria	20
Package and color	5

Obviously, it is very seldom, if ever, that a sample of ice cream, butter or cheese, is given a perfect score of 100. No one actually knows what perfection is and some allowance must be made for this. In general a sample receiving a score of 90 per cent or above may be considered excellent, while a score of 80 to 90 would be representative of a good quality of ice cream. A score card or yard stick for measuring quality similar to the above, has now been used for two years at the Eastern States Dairy Products Judging Contests and also at the Educational scorings at the Massachusetts and Connecticut Agricultural Colleges. At each of these educational scorings, about thirty-five commercial samples from all over New England were received. At these occasions the score card has proven its practicability and value in measuring quality factors in ice cream, provided a uniform system of grades and standards can be worked out. To start the ball rolling and open up an opportunity for exchange of ideas, the following plan is suggested:

FLAVOR OR PALATABILITY

The quality of the flavor of ice cream may be classified from the standpoint of palatability under four general groups:

1. Highly pleasing and desirable: Flavors rating 45 to 50 points.
2. Desirable flavors: Flavors rating 40 to 44.9 points.
3. Objectionable flavors: Flavors rating 35 to 39.9 points.
4. Foreign (off) flavors: Flavors rating 25 to 34.9 points.

1. Highly pleasing and desirable flavors: Rating 45 to 50 points

Ice cream that is especially fresh, clean, sweet and well blended in flavor, having the proper degree of sweetness and flavoring and having a certain creaminess or richness in flavor characteristic of the pleasing flavor and aroma of fresh sweet cream, shall receive a rating of 45 to 50 points.

Descriptive terms: Fresh, clean, creamy, well blended.

2. Desirable flavors: Rating 40 to 44.9 points

Ice cream that is fresh, clean, creamy and sweet in flavor, but high or low in sweetness or flavoring material, shall be given a rating of 40 to 44.9.

Descriptive terms: Too sweet, lacking sweetness, too high flavoring, lacking flavoring.

3. Objectionable flavors: Rating 35 to 39.9 points

This class includes ice cream that is free from foreign, (off) flavors but shows objectionable flavors, such as old cream, old butter, bitter, cooked, condensed or powdered milk, gelatin or unnatural flavoring or unnatural flavoring such as pronounced glucose. Such ice cream shall receive a rating for flavor of between 35 and 39.9 points.

Decsriptive terms: Old cream, old butter, old egg butter, cooked condensed milk, powdered milk, gelatin, unnatural flavoring, unrecognizable.

4. Foreign (off) flavors: Maximum rating 25 to 34.9 points

These include flavors, ordinarily termed foreign flavors (off) flavors which are distinctly disagreeable to the taste.

Ice cream showing salty, rancid, garlic, gasoline, disinfectant, unclean utensils or any other foreign (off) flavor distinctly disagreeable to the taste, shall be given a score of 25 to 34.9 points.

Descriptive terms; Salty, rancid, garlic, gasoline, disinfectant, unclean utensils, unrecognizable.

Ice cream with pronounced sour or other flavors bad enough to make it unsalable, shall be score 0 on flavor.

Note: Ice cream is never scored perfect in flavor, as no one knows what perfection is and 2 to 4 points leeway must be allowed. A score of within 2 to 4 points of perfect may therefore be considered excellent.

BODY AND TEXTURE

Class I: Rating 23 to 25

Ice cream receiving the rating of 23 to 25 must be firm, smooth and velvety in body and texture.

Descriptive terms: Firm, smooth, velvety.

Class II: Rating 20 to 22.9

Ice cream that is slightly fluffy, crumbly, icy, coarse, buttery, weak i. e. (no resistance), or soggy shall receive a rating of 20 to 22.9.

Descriptive terms: Fluffy, crumbly, icy, coarse, buttery, weak, soggy.

Class III: Rating 15 to 19.9

Ice cream that is sandy, pronounced soggy, buttery, icy, coarse, crumbly, weak, gelatin lumps, shall receive a rating of 15 to 19.9.

Descriptive terms: Sandy, soggy, crumbly, buttery, icy, coarse, weak, gelatin lumps.

PACKAGE AND COLOR

A. The suggested standard color for vanilla ice cream is the color of Guernsey or Jersey cream during the month of June or July with allowance for deeper shades of color when eggs are used in the mix. However the different markets vary greatly regarding the degree of color desired in ice cream. In the case of vanilla ice cream these requirements vary from almost white to a deep egg yellow:

In order to meet these requirements for color in ice cream the following shall be used as a basis of rating on color:

1. Ice cream in order to receive the full rating of 5 points must be free from specks, unnatural or uneven colors.

2. Ice cream showing dirt specks, unnatural colors or uneven colors shall be cut not exceeding 2 points according to the degree of the defect.

Descriptive terms: Dirt specks, unnatural, uneven.

B. *Package:* Ice cream receiving the full rating of 5 points must be neatly and solidly packed in clean, non-rusty cans and tubs, the cans being provided with parchment paper circles over the top.

Ice cream packed in unclean, rusty cans, or not provided with parchment circles shall be cut not exceeding 2 points according to the degree of the defect.

Descriptive terms: Unclean, rusty cans, no parchment.

With this exposition before you the following are some of the many questions that might be raised and discussed:

1. Is it wise to have a score card based on 100 points as perfect and considering only flavor, body and texture; and color and package for student contest work and another so-called commercial card considering not only the above items but bacteria as well?

In our opinion one complete score card for ice cream is enough. The need of an ice cream score card for students' judging work is very acute. In these contests students score ice cream on flavor, body and texture and color and package. It seems that there is need for a score card in the commercial field especially in educational scoring work. Such a score card undoubtedly should consider a measure of how the ice cream has been produced and handled. While the number of points that should be allotted to flavor, body and texture, and color and package together with a system of grades and standards for same are the most pressing things that need to be decided, there seems to be no good reason why bacteria should not be considered on the same score card. The essential thing to decide is the number of points to be allowed for each item on the score card and then adopt some system of grades as a trial until such time as further experience warrants a change.

2. Should body and texture be considered separately and given separate scores?

The suggestion has been made that body and texture are two different things and that they should be separated. We feel it is so difficult to draw the line on what is meant by body and what by texture that it would lead to confusion to separate them.

In a recent survey of college diary departments 12 are in favor of scoring body and texture together while only 3 are opposed and 3 non-committal.

3. In the score card suggested are the points apportioned correctly among the different items? Are items mentioned that should not be considered or are there items not mentioned that should be considered?

It seems advisable to allow 5 points for color and package. These are of relatively minor importance but certainly ice cream in a rusty or dirty container or ice cream of very unnatural color should not receive a perfect score.

It is doubtful if it can be argued that any kind of bacteria even the acid formers are beneficial in ice cream. If this is true ice cream with high bacteria count should not get as high a score as ice cream of low bacteria count. There are those who seem to feel that not enough is known about the bacteriology of ice cream to warrant its consideration on the score card. As the writers see it it is simply a question as to whether our bacteriologists know how to accurately sample ice cream and make an accurate bacteria test on the sample.

A few years ago about the same proposition confronted us in the case of market milk. Bacteria counts were considered unreliable. Would not including an allowance for bacteria on the score card help materially to stimulate research along the line of reliability of bacteria counts for ice cream. It is apparent that including bacteria count on the milk score card helped materially to stimulate investigation on standard methods of bacterial analysis for milk.

With regard to grades and standard for bacteria it will require considerable amount of experimental work, as a large number of bacteria counts made according to an approved method on a number of samples of ice cream are essential in deciding this point.

Baer of Oklahoma, and Olsen of Kansas have scored a large number of samples of ice cream on which bacteria counts have been made. In their work, samples showing 20,000 or less cubic centimeters have been given a perfect score of 20 points. The

following system of grades that we are suggesting does not differ very materially from the one used by Baer and Olsen. For 20,000 bacteria or less per cubic centimeter allow 20 points. Deduct $\frac{1}{2}$ point for each 10,000 until 100,000 is reached, then deduct 1 point for each 25,000 until 200,000 is reached, after which deduct 2 points for each 50,000 until 500,000 is reached afterwhich the score shall be 0.

The remaining question is, should more weight be given to flavor and less to body and texture. It is true that most of the large plants have a better control on body and texture than is possible for flavor and most of the ice cream from the large plants is perfect, or nearly so, in body and texture. However, the large number of commercial samples submitted to our educational scorings last winter show all sorts of body defects and convinces us that the smaller plants, especially, have not solved the problem of turning out ice cream of uniformly good body and texture. The consumer of ice cream will put up with a flavor that at best might be termed only fair, while coarse, icy or buttery ice cream will draw complaint at once. The writers of course feel that more weight should be given to flavor than body and texture, the above statements are made simply to argue the point that 25 points are not much less than should be allowed for body and texture.

4. Are the flavors properly classified and the cuts allowed for each group what they should be? Are there other off flavors that should be included in any of the classifications?

The matter of the number of points to cut for any defect does not seem so important as some of the previous questions. The important thing is to have some standard to follow and then have everybody stick to it. We have set our lowest flavor score on any ice cream that can be considered salable, from the standpoint of flavor, at 25 points. Ice cream of such poor flavor as to obviously make it unsalable is score 0 on flavor. This seems much better than allowing a range of from 0 to 25 points for the fourth class of flavors as it does away with the element of guess work to a large degree. To illustrate, a sample might be slightly salty but not bad enough to really class it as unsalable. If a free range of from 0 to 25 points was allowed for this defect the

judge might score it 10 while the student might score it 20. One guess might be as good as the other. Following our suggestion both judge and student would know the sample must score 25 to 34.9 points. This scheme does not solve the problem of differences in judgment of individuals as to what constitutes a flavor bad enough to make ice cream unsalable nor does there seem any way of solving this problem. However the matter is not serious as most ice cream is good enough to eat and if a sample is really bad it is likely to be so bad as to leave no doubt in the minds of any one as to its salability.

5. Are body and texture defects properly classified? Are there other defects that should be included?

It is not likely that ice cream will have a sufficiently poor body and texture to make it unsalable hence to avoid too wide a range of cuts for class 3 defects 15 points is considered the lowest possible score on body.

6. Should color receive any consideration on the score card and is the maximum cut of 2 points too large or too small?

It is felt that a fairly wide range should be allowed in scoring color of ice cream. The maximum cut of 2 points is intended to apply only to extreme cases where the color is very unnatural for the flavor with which it is used.

7. Some may criticize this card because it does not seem to consider fat and total solids or food value. The writers have been guided by what seems to them to be a wise suggestion coming from two well known ice cream authorities. The suggestion is, let fat and solids be governed by the state standard in the state in which it is made. Ice cream submitted for scoring would, of course, be tested for fat and total solids and the report sent to the manufacturer with the score scard. Ice cream that was below the state standard would be thrown out of any contest as it would not be legally called ice cream. In states having low standards for butterfat, if ice cream was made down close to the standard, it would be shown up on body and texture and flavor, unless by chance the solid not fat content was well reenforced, in which case, the ice cream should not be cut for lacking in food value. In general, there are no state total solid standards. Fat standards

differ so widely in different states that if fat and solids was given its allotment of points on the score card, we never could nationally reach an agreement as to what per cent of fat and solids constitutes perfection, and hence a base below which deductions would be made.

It is also a question in our minds if ice cream from a food value standpoint does not resemble cheese and butter more closely than it does milk. No direct consideration of food value is given on the butter and cheese score cards. Results of our educational scoring indicate that extreme variations in ice cream composition, especially total solids, is not the common thing. A variation of 2 to 4 per cent even though it might not show up in body and texture or flavor, is probably not at all serious from the standpoint of food value of ice cream.

The argument of simplicity, i.e., making the score card as simple as possible and doing away with "ornamentation" items is an important one and one that we have endeavored to bear in mind in making these suggestions.

SOME FACTORS AFFECTING THE KEEPING QUALITY OF WHOLE MILK POWDERS[1]

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It is generally known that whole milk powders of the past have not been endowed with the good keeping quality of skimmilk powder. The reason for this difference is the presence of butterfat in large amounts in the whole milk powder. These powders contain on the average from 26 to 29 per cent fat. The fat is subject to deterioration which may render the powders unsalable when this deterioration reaches a point where the flavor and odor are affected.

There are two different types of deterioration of fat which are noticeable in milk powders, namely, one associated with oxidation of the fat, producing tallowiness, and one associated with hydrolytic decomposition resulting in the liberation of butyric and other volatile acids. The fat affected by hydrolytic deterioration is rightly termed rancid.

Other off flavors may occur in whole milk powders which are also common in skimmilk powders. Decomposition such as staleness, mustiness, and storage flavors are not necessarily associated with butterfat. These defects are not powder defects but rather defects due to age, package, storage temperature, and time stored.

Earlier whole milk powders deteriorated in a very short time. Present day whole milk powders also occasionally deteriorate quickly. The writers have encountered typical cases of rancidity in powders which were less than five weeks old. Again,

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powders made by the same company have kept many months without serious defects. The quality of the fresh milk and the treatment of the milk prior to powdering have a decided effect upon the keeping quality of the finished product. Where the liquid milk is subjected to high temperatures of pasteurization (175° to 180°F.) little danger from rancidity need be expected. The lipase enzyme, which may be termed the cause of rancidity of butterfat, is rendered inactive at this temperature.

Tallowiness of the butterfat in milk powders is due to oxidation of the fat. Air, especially hot air, is a prominent factor in producing this decomposition. Earlier investigators (1) found that powders made by the drum process exhibited superior keeping qualities to powders made by the spray process. The writers (2) made note of the same conditions, but the drum-made powder studied was made from partly skimmed milk and contained but one-half as much fat as the spray-made powder. The writers expressed the opinion that the more rapid development of tallowiness in spray-made powders may be due to the peculiar structure of the powder grains. It was found that many, and in some cases each individual spray-made powder grain contained an air cell, thus exposing more of the fat to air. The onset of tallowiness was also found to be in proportion to the size of the air cell. No lipase activity or rancidity was noticed in the drum-made powder. The Storch test made on the drum powders studied showed that the fresh milk had been heated to at least 172°F.

PLAN OF EXPERIMENT

It was the purpose of this experiment to make a study of some of the factors affecting the keeping quality of certain whole milk powders and to compare the keeping quality of powders made by the various processes. Three types of powders were studied—one type (Klim) representing the pressure spray system known as the Merrell-Soule System, another type (Creamon) representing the centrifugal spray system controlled by the Dick Patents, and Creamora A representing the drum system and made by the Dry Milk Company of New York.

The factors studied were as follows:

1. The effect of increasing the moisture content of the powders on the keeping quality.
2. The effect of type of containers on the keeping quality.
3. The effect of storage temperature on the keeping quality.
4. The effect of time in storage on the keeping quality.

The samples were stored at three temperatures, namely, 4°C. (refrigerator), 20°C. (room), and 37°C. (incubator).

Scoring of the samples took place after they had been in storage three, six, nine, and twelve months. A sufficient number of samples was stored to permit the opening of a new sample at each scoring. In all 216 samples were used in the experiment and scored according to flavor and odor. No attempt was made to place a numerical score on the samples, but a complete set of notes was kept of the types of deterioration and the degree of decomposition noted.

The moisture content of the powders before and after exposing to damp air is shown in table 1. The moisture was increased by placing the powders in flat pans and exposing them for several hours at room temperature to an atmosphere nearly saturated with moisture. The samples were then packed into 2-ounce opaque glass jars. It required 39 grams of Klim, 20 grams each of Creamora A and Creamon to fill the jars with moderate packing. These weights indicate that Klim powder either consisted of smaller individual particles or contained a smaller air cell within the particles.

It is possible that deteriorations due to the treatment received by these powders in this experiment should not be attributed solely to the increased moisture content but partly to the exposure to moist air, inasmuch as the total moisture content of the exposed powders was not excessively high.

The deteriorations observed in table 2 show the deleterious effect of moisture and oxidation. It will be noticed that all of the samples stored at room temperature for three months became either off-flavored or discolored. Creamon powder exhibited the most pronounced tallowiness, followed by Creamora A. At six months all showed evidence of deterioration.

TABLE 1

The effect of exposing powders for three hours in an atmosphere having a humidity of 70 per cent

POWDER	ORIGINAL MOISTURE	MOISTURE CONTENT AFTER EXPOSURE
	per cent	*per cent*
Creamon	2.98	4.31
Klim	2.57	4.60
Creamora A	2.56	4.56

TABLE 2

The influence of increased moisture on the keeping quality of the powders studied

TIME STORED	20°C.	4°C.	37°C.
	Creamon powder		
months			
3	Very tallowy; unfit for use	Tallowy but not as bad as the 20° sample	Very tallowy, discolored and hard
6	Flavor stale	Stale, flat	Very tallowy, discolored and hard
9	Stale and tallowy	Stale, tallowy	Very tallowy, discolored and hard
12	Tallowy, hard	Stale, hard	Very tallowy, discolored and hard
	Klim		
3	Yellow color. Flavor good	Flavor good	Very tallowy, discolored and hard
6	Odor good; flavor slightly off	Odor dead, flavor flat	Very tallowy, discolored and hard
9	Stale and hard	Flat, though slightly stale	Very tallowy, discolored and hard
12	Tallowy and hard	Tallowy and hard	Very tallowy, discolored and hard
	Creamora A		
3	Very slightly tallowy	Good	Very tallowy, discolored and hard
6	Slightly tallowy	Odor of ice box	Very tallowy, discolored and hard
9	Stale, not usable	Flat	Very tallowy, discolored and hard
12	Stale	Tallowy	Very tallowy, discolored and hard

Samples from the same powders stored at lower temperatures showed slightly better keeping quality except in case of Creamon powder. After three months time all showed evidence of deterioration.

Deteriorations were more pronounced in the samples stored at 37°C. in the incubator than at either of the other temperatures. All of these samples became hard, yellow, tallowy, and utterly useless for any purpose. The deterioration in every sample had proceeded to the limit in three months time. No distinction could be made between the extent of the deterioration of the different powders. The higher temperature of the incubator greatly favored and accelerated the decomposition due to the higher moisture.

EFFECT OF TYPE OF CONTAINERS ON KEEPING QUALITY

To note the influence of containers on the keeping quality of whole milk powders several types of containers were employed. Small opaque glass jars with metal screw tops were used as well as "Sealright" paper containers, a plain paste board container, and "Doubletite" tin containers, both plain and "lacquered." The lacquered tin containers were made by coating the inside of the "Doubletite" containers with shellac to keep the powders from coming in contact with the metal.

THE KEEPING QUALITIES OF POWDERS STORED IN OPAQUE GLASS JARS

The opaque glass jars were used as check containers for the other experiments because of the freedom of glass from catalyzers and metal. The keeping quality of the powders stored in these containers is shown in table 3.

All powders kept well up to three months except the samples held at 37°C. These became hard, discolored, stale, and tallowy.

Slight off flavors were observed in the samples after six months in storage at 20°C. The degree of deterioration was slight, however.

The samples held for nine months and one year respectively, showed pronounced deterioration and were in general not usable or salable.

TABLE 3

The keeping quality of whole milk powder stored in opaque glass jars

TIME STORED	20°C.	4°C.	37°C.
		Creamon powder	
months			
3	Good	Good	Very tallowy, discolored (yellow) hard
6	Flavor slightly bitter, off	Slightly stale	Very tallowy, discolored (yellow) hard
9	Stale, not salable	Stale, tallowy	Very tallowy, discolored (yellow) hard
12	Tallowy, hardened	Flavor musty, stale; less soluble than that kept in tins	Very tallowy, discolored (yellow) hard
		Klim	
3	Good	Good	Very tallowy, discolored and hard
6	Very slightly off	Odor of ice box. Flavor good	Very tallowy, discolored and hard
9	Stale, hard	Slightly off.	Very tallowy, discolored and hard
12	Tallowy, hard	Stale, bad flavor (not as soluble as that in tins)	Very tallowy, discolored and hard
		Creamora A	
3	Good	Good	Very tallowy, discolored and hard
6	Odor good; flavor slightly dead	Storage odor	Very tallowy, discolored and hard
9	Slightly tallowy	Stale, musty	Very tallowy, discolored and hard
12	Flat, stale	Flat flavor (not as soluble as that in tins)	Very tallowy, discolored and hard

In general it may be said that this type of container was not suitable for storing whole milk powder, since it was not airtight. To prevent the entrance of air when using this type of container the covers should be sealed on with melted paraffine.

THE INFLUENCE OF "SEALRIGHT" CONTAINERS

Table 4 shows the effect of the "Sealright" paper containers on the keeping quality of the various powders. The Creamon and

TABLE 4

The influence of "Sealright" paper containers on the keeping qualities of whole milk powder

TIME STORED	20°C.	4°C.	37°C.
		Creamon	
months			
3	Good	Odor off, slight moldy flavor	Very tallowy, caked hard (yellow)
6	Good	Odor dead; flavor stale	Very tallowy, caked hard (yellow)
9	Stale, tallowy	Ice box odor, stale	Very tallowy, caked hard (yellow)
12	Slightly tallowy and stale	Flat, storage flavor; odor gone	Very tallowy, caked hard (yellow)
		Klim	
3	Good	Flavor, odor good; slight change in physical structure	Hard, very rancid; green mold present
6	Good	Storage flavor, flat	Very tallowy, hard and brown
9	Slightly tallowy	Flat, unsalable	Very tallowy, hard and brown
12	Stale, individual particles hard	Musty	Very tallowy, hard and brown
		Creamora A	
3	Slightly tallowy	Musty flavor	Brown and tallowy but not as hard as Klim
6	Odor of stale pastry	Odor of ice box; flavor stale	Brown and tallowy but not as hard as Klim
9	Slightly tallowy	Flat, unsalable	Brown and tallowy but not as hard as Klim
12	Stale, individual particles hard	Musty flavor; odor gone	Brown and tallowy but not as hard as Klim

Klim powders kept exceedingly well at the end of six months at room temperature. Creamora A showed deterioration at the first examination after three months in storage. The deterio-

ration was characterized by slight tallowiness and at six months by staleness.

"Sealright" cans are evidently too porous to permit their use in damp places such as refrigerators, where the moisture content is usually high. None of the powders kept well in "Sealright" containers when held under this condition. The original flavor and odor of the powder were lost and musty and storage flavors had appeared at the time of the first examination. Room temperature storage gave considerably better results than either refrigerator temperature (where humidity was high) or incubator temperature (37°C.).

INFLUENCE OF PLAIN PASTE BOARD CONTAINERS

The results with the plain paste board containers are shown in table 5. These containers do not have as thick walls as "Sealright" containers, otherwise there is little difference in general appearance. Practically the same conditions were noted in the use of these containers as in the use of "Sealright" containers, for the first six months. Creamon and Klim powders did not deteriorate appreciably at room temperature. Creamora A showed signs of deterioration at the first examination. These containers are also too porous to permit their use in refrigerators of humid places at either high or low temperatures. Fat leaked through the sides of these containers containing Creamora A powder stored at 20° and at 37°C. The deterioration of fat in these samples was extremely noticeable. The cause of the leaky fat will be discussed under "the comparison of keeping quality of the various powders."

The characteristic musty and storage flavors and odors were noticeable in powders stored at 4°C. The musty odors disappeared in some instances at the end of one year.

INFLUENCE OF PLAIN TIN ("DOUBLETITE") CONTAINERS ON KEEPING QUALITY

The plain tin container gave excellent results, as shown in table 6. Very little air, if any, could gain access to the powder

because of the tight fitting cover. The Creamon and Creamora A powders after three months at 20°C. were scored off slightly on odor, and at six months a trifle on flavor. After six months to one year there was little criticism to make of any powders stored at

TABLE 5

Influence of paste board containers on keeping quality of whole milk powders

TIME STORED	20°C.	4°C.	37°C.
		Creamon	
months			
3	Good	Good	Badly oxidized; hard, yellow, tallowy
6	Good	Dead odor; off flavor	Badly oxidized; hard, yellow, tallowy
9		Dead odor, stale	Badly oxidized; hard, yellow, tallowy
12	Stale; particles hard	Flavor flat, storage odor	Badly oxidized; hard, yellow, tallowy
		Klim	
3	Good	Odor of paper; flavor good	Hard, yellow, tallowy
6	Flavor not as good as "Sealright"	Flavor dead	Hard, yellow, tallowy
9	Stale, hard	Good	Hard, yellow, tallowy
12	Stale, particles hard	(lost)	Hard, yellow, tallowy
		Creamora A	
3	Moldy (yellow mold) Slight butter flavor	Slightly stale; musty flavor	Brown but not very hard. tallowy
6	Flat	Musty odor; flavor stale but not as bad as "Sealright"	Yellow, hard, tallowy
9	Stale, unsalable	Musty flavor	Yellow, hard, tallowy
12	Stale, particles hard	Flat	Yellow, hard, tallowy

room temperature except the Creamora A sample which showed a slight staleness after 9 months and tallowiness after one year in storage.

The samples stored at 4°C. came out after one year's time in salable condition. They had lost somewhat their original fresh

flavors and odors during that time, but none exhibited any serious defects.

TABLE 6

Influence of plain tin "Doubletite" containers on keeping quality of whole milk powders

TIME STORED	20°C.	4°C.	37°C.
		Creamon	
months			
3	Very slightly off in odor; flavor good	Good	Tallowy but not baked hard nor discolored as in other containers
6	Flavor slightly off	Odor dead; flavor stale	Tallowy and slightly brown in color
9	Odor and flavor good (particles hard)	Odor and flavor good (particles hard)	Tallowy but not baked hard nor discolored as in other containers
12	Flavor good (particles hard)	Slightly flat but good	Odor and flavor bad; color white
		Klim	
3	Good	Good	Color normal: not hard; flavor off
6	Odor slightly stale; flavor good	Odor good; flavor good	Not discolored; odor slightly tallowy; flavor slightly tallowy
9	Good	Salable	Not brown nor hard; fair condition
12	Flavor good (particles hard)	Odor gone; flavor good; salable	Color normal; odor and flavor bad
		Creamora A	
3	Slightly stale flavor; odor good	Good	Not colored but slightly tallowy
6	Odor good; flavor dead	Good	Odor slightly tallowy; not yellow or hard; flavor slightly tallowy
9	Stale	Fairly good	Odor slightly tallowy; not yellow or hard; flavor slightly tallowy
12	Tallowy	Lost	Color white; odor and flavor bad

An outstanding characteristic of the plain tin "Doubletite" container was the protection offered the powders from heat and

oxidation. All powders in the other containers thus far studied, when stored at 37°C., became hard, tallowy, stale, and highly discolored. The color of some powders was deep brown. With only one exception the powders stored in this container at high temperatures came out at the end of a year without hardness or change in color. The color of all but one was as good as those stored at low temperatures. The flavor was affected in most cases, however.

From the data of this experiment the value of "Doubletite" or other perfectly tight tin containers for use in the tropics can be appreciated. No containers mentioned previously gave satisfaction under tropical conditions. The excellent keeping quality of powders stored in this tin container was undoubtedly due to the absolute exclusion of air and moisture.

INFLUENCE OF "LACQUERED" TIN CONTAINERS ON KEEPING QUALITY

There are tin containers on the market which are coated on the inside with a very thin coat of lacquer. Being unable to obtain any lacquered "Doubletite" containers a thin coat of shellac was applied to the inside of some plain "Doubletite" containers similar to the ones used in the previous experiment. The shellac was permitted to dry thoroughly before the powder was placed in the tins.

The shellac afforded no improvement over the plain tin containers as shown by the data in table 7. In fact the shellac had a detrimental influence. Up to six months in storage at room and low temperatures there was not much difference between plain tin and the shellacked tins, but after six months some of the powders in the "Shellacked" containers developed a cheesy odor. This was noticeable in many instances and the powder so affected was rendered unsalable and unfit for human use.

The same protection against hardness, extreme tallowiness, and discoloration was afforded by the shellac lined tin as was noted when the plain tins were used. With one exception all powders examined after being in storage for one year at 37°C. were free from color change. This exception as well as the exception in the

TABLE 7

The influence of tin (lacquered) containers on the keeping quality of whole milk powders

TIME STORED	20°C.	4°C.	37°C.
	Creamon		
months			
3	Slightly off	Good	Cheesy bad odor, not yellow, nor hard
6	Cheesy odor, evidently protein decomposition	Flavor slightly cheesy; odor dead	Tallowy, not hard nor discolored
9	Cheesy odor; cheesy flavor	Cheesy odor and flavor	Not hard nor yellow but very cheesy odor
12	Cheesy, bad	Slightly rancid, but fair	Brown but not hard
	Klim		
3	Good	Good	Off, not hard nor discolored
6	Odor good	Flavor nearly gone	Not colored; flavor and odor slightly stale
9	Stale, probably due to lacquer	Good but odor of lacquer	Slightly stale; not hard nor brown
12	Stale, off-flavored	Slightly stale but good; more soluble than others	Color good; odor and flavor bad
	Creamora A		
3	Off odor and flavor cheesy	Good	Not colored, but cheesy; slightly tallowy
6	Odor good; flavor dead	Odor good; flavor good	Flavor and odor slightly tallowy; not yellow nor hard
9	Off in flavor	Off, due to lacquer	Color white; flavor and odor bad
12	Slightly cheesy probably due to lacquer	Slight storage flavor but not as bad as paper containers; more soluble than in paper containers	Color white; odor and flavor bad

plain tin container was found in the Creamon powder. This powder ordinarily contained more air within each particle than either of the other powders studied. It may have been possible that the covers admitted some air in the cases where exceptions were noted.

THE INFLUENCE OF STORAGE TEMPERATURE ON KEEPING QUALITY OF WHOLE MILK POWDER

It was evident from the start that an extremely high temperature (37°C.), such as was experienced in this experiment, was very deleterious to keeping quality. This was augmented by the presence of air. When air could be excluded, as in case of powders packed in "Doubletite" tins or packed in vacuum, practically no browning or discoloration was experienced. The vacuum tests consisted of samples of each powder packed in porcelain jars without covers. These jars of powders were then placed in a desiccator upon which a 29-inch vacuum was drawn. The vacuum samples were discontinued after six months since no browning had been noticed. The tin containers were the only ones used which could be recommended for tropical use. Yet in these tins pronounced tallowiness was noticed in many instances after three months due to its heat and the air within the package. The other containers were useless at this temperature.

Many manufacturers have placed emphasis on the injurious effects of refrigeration on keeping quality of whole milk powders. This was borne out clearly in all cases except when powders were stored in airtight containers. Paper containers and others through which air might enter cause the powders to lose their fresh flavors and odors and become flat in some instances and in other instances to assume musty or storage odors and flavors. Tin containers kept the powders in good condition. In several instances salable and sweet powders were in evidence after a year's storage.

Not only did the powders lose their flavor when stored in paper containers at this temperature but the solubility of the powder was reduced considerably. This was especially true of the Cream-

ora A powder. It appeared to be tough and dough-like when attempts were made to dissolve it in the mouth.

Excellent results were obtained from storing powders in airtight containers, at 20°C. Creamora A powder stored in pasteboard containers leaked fat after three months' storage and became off flavored. Creamon and Klim kept better in paper containers when stored at room temperatures than they did when stored at low temperatures.

INFLUENCE OF TIME

The time factor was clearly shown to be a prominent factor as was expected. The longer the time stored the greater were the deteriorations in most cases. There were cases where a slight decomposition was recorded after six months in storage which were not noticed after nine months or one year. Occasionally certain off flavors seemed to volatilize in time, but this was not generally noticed. Time of storage, when coupled with the other factors, bore a vital relation to the solubility of the powders, as well as to the keeping quality.

COMPARISON OF THE KEEPING QUALITY OF THE POWDERS STUDIED

A comparison of the keeping quality of powders made by different processes would be meaningless without first calling attention to the source of milk from which powders are derived. To make a comparison of the effect of the processes of manufacture on the keeping quality, the milk from which the powder is derived should be from the same general source. This condition did not exist in this experiment. The milk entering the Creamora A (drum) and the Klim (pressure-spray) was produced in the eastern states from different sources, while the milk entering the Creamon powder (centrifugal spray) was produced in one locality in the middle west. A poor keeping powder is not necessarily indicative of poor raw milk supply. The sources of milk, the process of manufacture, time and conditions of storage are all contributing factors. All of the samples of powders were stored under iden-

tical conditions in this experiment so the contributing factors in the comparison of the various powders must be limited to the source of milk and the process of manufacture.

Early investigators and the authors have made statements which intimated that drum-made powder had superior keeping qualities over spray-made whole milk powders. The previous observations made by the authors were made on Dryco, a partly skimmed powder. The evidence from the present experiment showed that Creamora A (drum) did not exhibit keeping qualities superior to Klim (spray). In fact it was plainly inferior at the end of nine months and one year. The keeping quality of Creamon (spray) and Creamora A (drum) were not far different. In some cases Creamora A was slow to exhibit defects but in time the defects were plainly evident. The only conditions under which the Creamora A powder excelled in keeping quality were when it was stored at low temperature and in airtight containers. Creamon exhibited the poorest keeping quality when the moisture was increased. This was probably due to the high air content of this powder, since the increase in moisture was not as great in that powder as in the others studied.

In plain tins the Klim powder excelled in keeping quality. In these containers outside air was excluded so the only air present was the air within the particle in the form of an air cell, or the air surrounding the particles. As already noted, the particles of Klim powder permitted closer packing than the other powders.

Inferior keeping quality of Creamora A powder in paper containers was evident in most cases. It was plainly seen that the fat leaked from the powder through the pores of the plain pasteboard container to the outside when stored at 20° and 37°C., and a high degree of deterioration usually accompanied this occurrence. This leaking of fat was very likely due to the high degree of heat used in drying this powder. This high heat tends to decrease the stability of the hydrophilic colloids which are necessary to keep the fat dispersed.

The spray powders changed somewhat in physical condition during the experiment. All powders in the moisture experiment were nearly insoluble at the time of the last scoring. Samples

held in paper containers, especially those at low temperatures, decreased in solubility. Whenever pronounced tallowy flavors were evident the solubility was low.

Minute hard particles, similar to crystals, were noticed in almost all the samples at the expiration of the experiment. This condition suggested the presence of crystals of milk sugar, although microscopic examination revealed nothing unusual in the appearance of the individual particles. There was a noticeable decrease in solubility of the individual particles when observed under the microscope. Fresh spray powder disintegrates rapidly when a drop of water is placed on the particle on a slide. Water dropped on the powder on a slide at the close of the experiment showed very slow disintengration of the particle in most instances. Mild solutions of NaOH caused rapid disintegration of the individual particles, leaving the air cells which are found in the spray powders free to float around in the liquid. A few of the air cells broke, but the number which broke did not compare with the number which broke when the fresh powder dissolved in water. The surface tension of the air cell apparently increased greatly with age.

Powders which were subjected to the high temperature during storage and which had become brown and hard were insoluble in water and exhibited but very little disintegration when a drop of alkaline solution was dropped on a slide containing the powder grains.

The rapidity with which powder grains disintegrate in cool water and in weak alkaline solutions may be used as a qualitative index of their solubility.

SUMMARY

A study of the keeping quality of 216 samples representing three types of whole milk powders is described as follows:

Powders whose moisture content was increased by exposing the powders to moist air exhibited very inferior keeping qualities. Oxidation of the fat, giving rise to a tallowy odor, was the cause of deterioration.

Containers in which the powders were stored greatly influenced the keeping quality. Containers permitting the entrance of air proved to be useless for long time storage. Deterioration was very pronounced in the samples stored in these containers, the extent of deterioration was usually more marked in case of the Creamora and Creamon A powders. Klim powder is not exposed to as much air as either of the other powders in the package. It has smaller individual powder grains which permit closer packing than Creamora A and Creamon, and the air cell when present within the powder grain is much smaller than that of the other spray powder, Creamon.

Superior keeping quality was observed in samples of powders stored in tin "Doubletite" containers. These containers provided for absolute exclusion of outside air and moisture. In some cases samples were practically unchanged after a year in storage. Protection against discoloration, due to high storage temperature, was afforded when this type of container was used. This was not the case when other containers were used. Lacquered tin containers afforded no better protection than the unlacquered. In most instances the keeping quality was inferior to powders stored in the unlacquered tins.

Pasteboard containers proved to be unsatisfactory, since air and moisture gained access to the powder. Tallowy and musty flavors were the main deteriorations observed.

The temperature at which the powders were stored proved to be an important factor. Not a great deal of difference was observed between powders stored at 4° and 20°C., but a great difference was observed when powders were stored at 37°C. Most of these powders deteriorated very rapidly at this temperature and became very hard and discolored. The powders stored in the tin containers "Doubletite" did not become discolored, and the deterioration was not so pronounced as in cases where other containers were used.

The influence of the time of storage depends on factors such as type of containers used and temperature of storage. Powders stored in opaque glass containers with screw tops and those in paste board containers showed signs of deterioration after three

Standing somewhat contradictory to these results are those of Hart, Steenbock and Hoppert (2), who observed positive calcium and phosphorus balances on three liberally milking cows. In these experiments alfalfa hay that had been cured under caps was fed as roughage. Replacing this alfalfa hay by green alfalfa seemed to even increase the retention of calcium and phosphorus. The suggestion is made that the process used in curing the hay was instrumental in preserving the "unknown factors affecting calcium assimilation." They stage that "in harmony with previous observations that green plant tissue contains more than dried plant tissue of some substance favoring calcium assimilation."

These same authors (3) have shown that milking goats are enabled to utilize more efficiently the calcium of the ration when the fresh green oat plant is fed in preference to oats straw; and that the curing of oat hay out of the direct sunlight aided in the retention of those qualities assisting calcium assimilation.

Later work by Hart, Steenbock, Hoppert and Bethke (4) has shown liberally milking cows to be losing calcium and phosphorus. In this case, alfalfa hay cured by four days exposure to the sun while in the windrow was fed. However, these losses were often slight and as the authors state, "could no doubt be maintained for a long time without serious results to the animal." When timothy hay was fed large losses of calcium and phosphorus were encountered. Adding steamed bone meal to the ration containing the timothy hay reduced these losses somewhat but did not make this ration on a par with that containing the alfalfa hay.

Meigs, Blatherwick and Cary (5), conclude from the results of metabolism experiments that the disturbance to the cow resulting from the separate collection of urine and feces as practiced in the metabolism experiments may interfere with the assimilation of phosphorus and nitrogen and more especially calcium. They point out that large calcium losses from the bodies of cows as reported in the various balance experiments are not to be explained on the basis of a wasting of the bones; because the losses of calcium and phosphorus have not been in

the same ratio as that in which these elements are found in the bones. Meigs (6) also points out the apparent impossibility of large losses of calcium as reported by Forbes.

TABLE 1

Data concerning cows used

	cow 111	cow 146	cow 154	cow 192
Breed	H.F.	H.F. (grade)	H.F.	H.F. (grade)
Date of birth	1/31/13	4/10/15	7/17/15	8/28/17
Previous lactations	5	3	3	1
1921				
Freshened prior to test	12/30/20	2/12/21	12/25/20	3/18/21
Live weight at start of test (pounds)	1273	955	1102	981
Day of lactation at start of test	195	153	200	117
Average daily milk production (pounds)	30.34	30.59	31.09	32.30
Type of ration, lactation and test	1:9	1:4	1:9	1:4
Month of gestation (test)	3	Not bred	3	1
1922				
Freshened prior to test	1/20/22	6/8/22	1/21/22	3/21/22
Live weight at start of test (pounds)	1330	1083	1135	1071
Day of lactation at start of test	175	44	181	111
Average daily milk production (pounds)	38.18	52.20	32.91	32.27
Type of ration, lactation and test	1:4	1:9	1:11	1:2
Month gestation (test)	Not bred	Not bred	1	Not bred

EXPERIMENTAL

Cows

Table 1 gives the essential data concerning the cows used in these experiments. They have been reared from weaning time on rations of the same type as those supplied to them in the 1921 balance trial. Their dams had also received rations of like character. The 4 cows used in this work have never tasted pasture grass. They have grown to maturity and have seemingly developed normally. The calves produced by them have

been normal. Although abortion has been more or less prevalent in the herd, kept in the same barn, this disease has never occurred among these 4 individuals. The average frequency of calving has been approximately every thirteen months. Two of them, nos. 111 and 154, are purebred Holstein-Friesian cows, while the other 2, nos. 146 and 192, are high-grades of the same breed. These animals were included in the group of 7 cows upon which mineral palatability tests were conducted by Forbes (7).

Although these cows were at their minimum live weight during the tests in both years, it could not be said of any of them that they were in a weakened, emaciated or unthrifty condition. Their appetites were keen and they entirely consumed the feed offered during the time covered in the data reported. Cow 192 in the 1922 experiment went off feed on the eighth day and was dropped from the experiment. However, after the administration of a physic and the withholding of two feeds, she was again able to consume a ration of the same type and amount as formerly. The data on this cow for the 1922 test only cover a period of seven days or that time during which she entirely consumed all her feed and seemed perfectly normal.

These cows had passed the period of maximum milk flow, but had not reached that stage in lactation of rapid decline. In the 1921 experiment the average daily milk production ranged from 30 to 32 pounds. In the 1922 experiment, cows 154 and 192 were producing approximately 32 pounds daily; while the daily production of cows 111 and 146 was 38 and 52 pounds, respectively. In the 1921 test, cow 146 was farrow; cow 192 was in the first month of gestation while cows 111 and 154 were in the third month. They were all farrow during the experiment of 1922 with the exception of cow 154, which had been bred one month previously.

Feeds

All feeds used in these tests were of the best quality, with the exception of the timothy hay in 1921, which had evidently been cut when quite mature. The clover hay fed in both experiments was of that year's crop, having been cut about one week pre-

vious to the preliminary feeding. This hay was cured in the sun for two days and was then spread out on the barn floor and allowed to dry thoroughly. However, it was in fit condition for storage in the mow when taken from the field. It was of choice quality and had retained remarkably well its original color. One point which we think worthy of mention is, that

TABLE 2

Composition of feeds—per cent (as weighed for rations), 1921

FEED	PHOSPHORUS	SULPHUR	CALCIUM	MAGNESIUM	NITROGEN
Clover hay	0.1825	0.1762	0.9736	0.3003	1.893
Timothy hay	0.0942	0.1196	0.2547	0.1166	0.646
Beet pulp	0.0600	0.2783	0.7070	0.3948	1.355
Corn	0.2174	0.1199	0.0144	0.1586	1.385
Wheat bran	1.3712	0.2137	0.0948	0.6674	2.575
Cotton seed meal	1.2241	0.4847	0.2110	0.6814	7.060
Oil meal	0.8863	0.3130	0.3292	0.5696	4.680
Water		0.0001	0.0066	0.0016	0.0002

TABLE 3

Composition of feeds—per cent (as weighed for rations), 1922

FEED	PHOSPHORUS	SULPHUR	CALCIUM	MAGNESIUM	NITROGEN
Clover hay	0.1738	0.1783	1.1136	0.3287	1.944
Timothy hay	0.1813	0.1536	0.2596	0.1067	1.035
Beet pulp	0.0702	0.2350	0.6734	0.3069	1.370
Corn	0.2137	0.1176	0.0109	0.1092	1.450
Wheat bran	1.5161	0.2188	0.1067	0.6010	2.250
Cotton seed meal	1.3851	0.4672	0.1837	0.7076	6.720
Oil meal	0.6995	0.3659	0.3322	0.5416	5.490
Gluten meal	0.5453	0.9430	0.0191	0.0400	10.560
Water		0.00005	0.0048	0.0017	0.00016

this hay had not been through the sweat of the mow. The corn gluten meal used in the 1922 trial was a specially prepared product and ran extremely high in protein. The corn was of a yellow pigmented variety and used in the rations in a finely ground state.

The feeds were mixed, sampled for chemical analysis, weighed and put in paper sacks before the beginning of the experiments.

Each was weighed and analyzed individually. The beet pulp was placed in a separate paper sack and fed mixed with three times its weight of water. The hays were cut into 6-inch lengths, weighed and sacked separately. The percentage composition of the feeds are given in tables 2 and 3. The analyses are here stated on the same moisture basis as that found to exist when the feeds were weighed for the rations.

Rations

Table 4 shows the rations used in these tests. These same type rations had been fed from the beginning of the lactation period,

TABLE 4

Daily rations used (pounds)

FEED	COW 111		COW 146		COW 154		COW 192	
	1911	1922	1921	1922	1921	1922	1921	1922
Clover hay	5.0	12.0	12.0	5.5	5.0	4.5	12.0	11.0
Timothy hay	5.0	2.4	2.4	5.5	5.0	6.75	2.4	
Beet pulp	8.25	6.0	1.8	8.25	8.25	10.95	1.8	2.75
Corn	6.0	3.6	2.4	8.25	6.0	6.75	2.4	
Wheat bran	3.0	3.6	2.4	2.75	3.0	2.25	2.4	3.30
Cotton seed meal	0.3	3.0	3.0	0.55	0.3		3.0	3.30
Oil meal, O. P.	0.3	3.0	3.0	0.55	0.3		3.0	3.30
Gluten meal		1.2						5.22
Nutritive ratio	1:9	1:4	1:4	1:9	1:9	1:11	1:4	1:2

and rations identical as to feeds and amounts for four weeks previous to the preliminary period. A mineral mixture consisting of steamed bone meal, precipitated calcium carbonate, flowers of sulfur and salt, had been fed for a period of eight months, ending forty-five days prior to the beginning of the 1921 trial. No mineral mixture has since been fed to these cows.

In 1921 period, cows 111 and 154 received the wide ration, the nutritive ratio of which was 1:9; and cows 146 and 192, the narrow ration, the nutritive ratio of which was 1:4. The wide ration cows each consumed the same amount of feed, which is also true of the narrow ration cows.

In the second trial, that of 1922, these cows received rations of unlike character and amounts. Cow 111, which had formerly received a wide ration (1:9) was given a narrow ration (1:4) cow 146 was changed from a narrow ration (1:4) to a wide ration (1:9). Cows 154 and 192 received rations of the same type as in the 1921 balance trial, only more extreme; the ration of cow 192 narrowed from 1:4 to 1:2. These changes had been made at the beginning of the lactation periods.

Salt was fed separately at the rate of 2 ounces per day and entirely consumed by all the cows. This amount hardly seemed adequate for cow 154 in the 1922 trial.

The water supplied the cows for drinking purposes was deep well water, but it was not from the same source both years. It was given to the cows in such amounts as they would drink, but a strict account was kept of the quantity consumed. Samples for chemical analysis were taken daily. This deep well or natural water was used in preference to distilled water, in order to maintain as nearly as possible the normal conditions under which these cows had been kept. In our opinion, it is no more essential to purify the water used in the rations than it is to purify the feeds, provided, of course, that the same care be taken in determining the intake of the elements contained therein.

Conduct of experiments

The tests were conducted in the same barn in which the cows were kept and in stalls in which they had previously spent a part of their lives. The stalls were big and roomy and afforded the cows sufficient liberty to lie down in comfort. The experimental day started at 8:00 a.m. The cows were weighed each morning as soon after 8:00 a.m. as was possible, before watering, but after milking and feeding. Milking, feeding and watering were each done twice daily; and in all respects an effort was made to adhere as closely as possible to the routine to which the animals had been accustomed.

Collecting, sampling, etc.

The methods used in collecting and sampling the milk and excreta were similar to those used in like experiments. The

daily samples were placed in cold storage and kept until the end of the experiment. They were then composited. For the 1921 trial 6 composite samples of milk, urine and feces were saved from the products of each cow; and for the 1922 trial, 3 composite samples were saved. The milk and urine samples were analyzed in their original condition; while the feces samples were air-dried and finely ground and then analyzed. Consequently all data for the amounts and composition of the feces here stated refer to the air-dried condition.

The 1921 experiment extended over a period of eighteen days, from July 15 to August 1.

The 1922 experiment extended over a period of twelve days, from July 22 to August 2, for all cows except cow 192.

Methods of analysis

Calcium. McCrudden method (Jour. Biol. Chem., 1911, x, 194). Titrating calcium oxalate precipitate with potassium permanganate.

Magnesium. McCrudden method (Jour. Biol. Chem., vii, 2).

Nitrogen. Kjeldahl-Gunning-Arnold method—Official.

Phosphorus. Official gravimetric method. Digesting sample with nitric and fuming nitric acids in the presence of sulphuric acid.

Sulfur. Modified Benedict method (Jour. Amer. Chem. Soc., xli, 10).

DISCUSSION

Calcium balances

Of the 8 calcium balances here reported, 4 are negative and 4 are positive. The 4 negative balances occurred with cows receiving the wide rations. These losses of calcium were less than 1 gram in 3 cases and in the fourth, the loss amounting to 3.6 grams daily, occurred with a milk production of 52 pounds per day. All of the narrow ration cows were found to be storing calcium. Three of these storages were approximately 4 grams and the fourth 8 grams. The plan of our experiment has not been such as to permit a definite answer explaining the cause

for this difference in calcium retention between the two groups of cows. But reasoning from the results derived from other experiments, we are able to offer a possible explanation.

Hart and associates (2) have shown the possibility of cows storing calcium when producing from 20 to 45 pounds of milk daily, when alfalfa hay which had been cured under caps was fed. When green alfalfa replaced the alfalfa hay, the storage of calcium was increased. These calcium storages when alfalfa hay, cured under caps, was fed seem to be contrary to later findings by the same authors (4), and also to the results of Forbes and associates (1). Hart and associates (2) suggest this difference in calcium assimilation has been due to the quality of the hay used. They ascribe to the alfalfa hay, cured under caps, some of the same powers influencing calcium assimilation as those proved to be present in the green alfalfa.

As previously mentioned, the clover hay used in both of our experiments was well cured fresh hay having been cut about one week prior to the preliminary feeding. It had retained remarkably well its original color and had not been subjected to overcuring in the sun. This hay was common to all the rations but used in much larger amounts in the narrow rations. In the light of the former work, just referred to, it may be reasoned that the storage of calcium by the narrow ration cows was due to the larger amounts of clover hay received by them, ascribing to this hay the presence of some organic factor assisting calcium assimilation. We merely offer this as a suggestion. There are other points to be taken into consideration in this connection, namely, the larger amounts of calcium and phosphorus contained in the narrow rations and that this type of ration furnished the greater part of its calcium in a leguminous roughage, the quality of the hay not being considered. Owing to the nature of our work we find it impossible to prove from the data the cause of this difference in calcium retention. But the balances here determined show that the narrow rations favored a greater calcium assimilation, than the wide rations; and that in accordance with the work of Hart and associates (2) it is possible for liberally milking cows to store calcium.

TABLE 5

Average daily balances of minerals and nitrogen (grams)

Cow 111, 1921, N.R. 1:9

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	12,632	35.98	24.38	56.74	39.34	196.97
Water	60,262		0.06	3.98	0.96	0.12
Total intake		35.98	24.44	60.72	40.30	197.09
Outgo						
Milk	13,763	11.86	3.20	13.30	1.63	56.16
Urine	13,394*	0.25	9.86	3.02	9.91	55.79
Feces	4,022	27.40	12.07	45.15	28.49	85.77
Total outgo		39.51	25.13	61.47	40.03	197.72
Balance		3.53	−0.69	−0.75	+0.27	−0.63

* Cubic centimeters.

TABLE 6

Average daily balances of minerals and nitrogen (grams)

Cow 154, 1921, N.R. 1:9

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	12,632	35.98	24.38	56.74	39.34	196.97
Water	61,232		0.06	4.04	0.98	0.12
Total intake		35.98	24.44	60.78	40.32	197.09
Outgo						
Milk	14,101	11.68	3.59	13.17	1.63	60.28
Urine	12,505*	0.16	8.37	1.62	7.68	38.98
Feces	4,368	24.94	12.65	46.17	31.90	89.45
Total outgo		36.78	24.61	60.96	41.21	188.71
Balance		−0.80	−0.17	−0.18	−0.89	+8.38

* Cubic centimeters.

TABLE 7

Average daily balances of minerals and nitrogen (grams)

Cow 146, 1921, N.R. 1:4

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	12,244	57.45	27.65	70.08	46.85	323.91
Water	69.283		0.07	4.57	1.10	0.14
Total intake		57.45	27.72	74.65	47.95	324.05
Outgo						
Milk	13,874	11.43	3.38	14.38	1.35	57.02
Urine	25,392*	0.24	12.12	1.52	7.53	163.94
Feces	4,439	50.34	14.12	54.58	39.07	95.99
Total outgo		62.01	29.62	70.48	47.95	316.95
Balance		−4.56	−1.90	+4.17	0	+7.10

* Cubic centimeters.

TABLE 8

Average daily balances of minerals and nitrogen (grams)

Cow 192, 1921, N.R. 1:4

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	12,244	57.45	27.65	70.08	46.85	323.91
Water	69.283		0.06	4.10	1.00	0.13
Total intake		57.45	27.71	74.18	47.85	324.04
Outgo						
Milk	14,693	12.61	3.68	13.15	1.59	62.92
Urine	15,368*	0.24	12.27	2.39	8.02	171.23
Feces	4,438	49.37	13.55	54.29	34.14	95.62
Total outgo		62.22	29.50	69.83	43.75	329.77
Balance		−4.77	−1.79	+4.35	+4.10	−5.73

* Cubic centimeters.

TABLE 9

Average daily balances of minerals and nitrogen (grams)

Cow 111, 1922, N.R. 1:4

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	15,785.4	72.93	39.74	90.82	56.21	438.45
Water	76,262.5		0.04	3.66	1.30	0.12
Total intake		72.93	39.78	94.48	57.51	438.57
Outgo						
Milk	17,314	13.41	4.48	18.13	1.97	78.62
Urine	21,464*	0.14	16.24	3.66	10.64	214.29
Feces	5,040	54.84	17.41	63.96	41.33	126.97
Total outgo		68.39	38.13	85.75	53.94	419.88
Balance		+4.54	+1.65	+8.73	+3.57	+18.69

* Cubic centimeters.

TABLE 10

Average daily balances of minerals and nitrogen (grams)

Cow 154, 1922, N.R. 1:11

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	14,134	34.50	25.82	65.50	34.65	206.50
Water	65,082		0.03	3.12	1.11	0.10
Total intake		34.50	25.85	68.62	35.76	206.60
Outgo						
Milk	14,925	12.15	4.05	15.06	1.67	64.68
Urine	16,004*	0.14	6.92	2.39	7.76	35.02
Feces	4,598	21.55	12.91	51.24	27.03	91.05
Total outgo		33.84	23.88	68.69	36.46	190.75
Balance		+0.66	+1.97	−0.07	−0.70	+15.85

* Cubic centimeters.

TABLE 11

Average daily balances of minerals and nitrogen (grams)

Cow 146, 1922, N.R. 1:4

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	14,220.2	43.60	26.28	62.49	37.06	238.37
Water	68,900		0.03	3.31	1.17	0.11
Total intake		43.60	26.31	65.80	38.23	238.48
Outgo						
Milk	23,678	18.83	5.89	27.28	2.89	95.03
Urine	11,778*	0.14	6.44	0.65	7.0	43.52
Feces	4,430	25.15	12.78	41.46	26.06	96.56
Total outgo		44.12	25.11	69.39	35.95	235.11
Balance		−0.52	+1.20	−3.59	+2.28	+3.37

* Cubic centimeters.

TABLE 12

Average daily balances of minerals and nitrogen (grams)

Cow 192, 1922, N.R. 1:2

	AMOUNT	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	NITROGEN
Intake						
Feed	13,095.2	76.35	49.91	73.73	48.88	580.57
Water	77,156		0.04	3.70	1.31	0.12
Total intake		76.35	49.95	77.43	50.19	580.69
Outgo						
Milk	14,636	11.28	3.84	14.84	1.86	66.80
Urine	34,632*	2.79	24.78	4.93	7.40	334.12
Feces	4,345	56.35	20.09	53.45	41.06	160.20
Total outgo		70.42	48.71	73.22	50.32	561.12
Balance		+5.93	+1.24	+4.21	−0.13	+19.57

* Cubic centimeter.

months in storage, while some powders stored in tin "Doubletite" containers showed scarcely no deterioration after one year in storage at 4° and 20°C. All powders stored at 37°C. showed pronounced deterioration at the time the first examination was made, or after three months in storage.

ACKNOWLEDGMENT

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THE METABOLISM OF CALCIUM, MAGNESIUM, PHOSPHORUS AND SULFUR IN DAIRY COWS FED HIGH AND LOW PROTEIN RATIONS

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For a number of years this Station has been studying the effects of wide and narrow rations on dairy cows. In 1921 and 1922 for the purpose of obtaining a more intimate knowledge of the utilization of the rations, digestion trials were conducted on four of the cows used in this study. As these animals had been restricted to winter rations all their lives, it seemed especially desirable that we should take advantage of the opportunity thus afforded and include in this work a determination of the balances of at least four of the more important mineral elements. The results of which are here reported, together with the nitrogen balances.

Forbes and associates (1) in an extensive series of metabolism experiments, have brought out the fact that the ability of the cow to utilize the inorganic constituents of the ration is much more limited than her ability to utilize the organic constituents. Their results particularly emphasize a limited ability of the liberally milking cow to utilize the calcium and phosphorus of the ration. The calcium balances were always negative with a milk production exceeding 10 pounds per day, while the phosphorus balances were usually so. Losses of these elements occurred regardless of an apparently sufficient supply of them in the rations, which included such roughages as clover and alfalfa hays. Larger losses of calcium were encountered during the feeding of timothy hay than during the feeding of clover and alfalfa hays.

Phosphorus balances

In considering the balances for this element, it must be remembered that the work of Hart and associates (2) has shown that the assimilation of phosphorus, like that of calcium, may be affected by the quality of the hay. Here, again, our use of fresh clover hay may have had some effect on the balances. However, in the 1921 data, we see that phosphorus was lost by all the cows, those fed on the narrow ration losing slightly more. These conditions are reversed in the data for the 1922 work in which 3 of the 4 balances are positive, the 1 negative balance of 0.5 gram occurring with a high level of milk production. In this latter experiment the narrow ration cows were storing phosphorus in amounts exceeding 4 grams, while the storage for the 1 wide ration cow whose milk production permits a comparison, was less than 1 gram per day. Here, then, we have a slight indication that the narrow rations would permit a larger storage of phosphorus. We are at a loss to explain why the difference between the phosphorus balances of the two years should have existed. The feeding of mineral supplements, which had ended forty-five days prior to the 1921 test, may have had some influence over the balances of that year.

Magnesium balances

Magnesium losses are shown in 3 of the 8 balances; these have all been less than 1 gram per day and all have occurred with the storage of nitrogen. Of the 4 positive balances of magnesium, 2 have occurred with nitrogen losses and 2 with nitrogen storage. The greatest amount of magnesium stored was 4 grams. There is little difference between the storages or losses of this element on the two different types of rations. The outgo of magnesium in the urine from each individual cow has seemingly been little affected by a change in the ration.

Sulphur balances

The balances for this element in the experiment of 1921 were all negative, the narrow ration cows losing slightly greater amounts

than the wide ration cows. In the 1922 experiment, the data show that there was an apparent retention of sulphur by all the cows, the amounts retained in each case were similar. The gain or loss of this element never exceeded 2 grams. It is apparent that the balances for sulphur have not been greatly affected by the type of ration consumed by the cows.

Nitrogen balances

In contrast to the extreme difference in the nitrogen intakes with the two types of rations, no marked differences are seen in the balances for this element. Six of the 8 balances are positive, the average daily storage varying from 3 to 19 grams. Two losses are noted, one amounting to less than 1 gram, occurring with a low nitrogen intake; and the other approximating 5 grams, occurring with a high nitrogen intake.

SUMMARY OF DISCUSSION

Eight balances of calcium, magnesium, phosphorus, sulphur and nitrogen are reported. Four of these balances were determined on cows receiving high protein rations, and 4 on cows receiving low protein rations.

The cows were all producing liberal quantities of milk, although in most cases, they had passed the period of maximum production. They were also at or near their minimum live weight.

The mineral content of the narrow rations was higher, especially in phosphorus and calcium.

All the cows receiving the high protein rations were found to be storing calcium, while those receiving the low protein rations were found to be losing this element. It is suggested that this difference in calcium storage was due to the larger amounts of clover hay contained in the high protein rations. The clover hay used was fresh hay that had not been subjected to an excessive amount of bleaching in the direct sunlight. The data here presented show the possibility of calcium retention with liberal milk production.

In the 1921 experiment, the phosphorus balances for the two groups of cows were somewhat similar, losses being noted in all cases. The results of the 1922 experiment indicate that the narrow rations, here used, would permit a greater phosphorus retention than the wide rations.

The magnesium, sulphur and nitrogen balances of the high protein fed cows show no marked difference from the corresponding balances of the low protein fed cows.

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A QUANTITATIVE DETERMINATION OF THE AMMONIA, AMINO NITROGEN, LACTOSE, TOTAL ACID, AND VOLATILE ACID CONTENT OF COWS' MILK

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A quantitative determination of the ammonia, amino nitrogen, lactose, total acid, and volatile acid content of 27 samples of commercial milk obtained from Baltimore dairies has been made within the past year. As this milk was to be used in a later bacteriological investigation, it was sterilized by autoclaving before being subjected to chemical analysis.

AMMONIA

Shaffer (1903) published a modification of the vacuum distillation method of Boussingault (1850). Shaffer stated that, although Boussingault had found no decomposition on distilling urea solution in a vacuum with either lime, sodium carbonate or bicarbonate at 50°C., and had concluded that there was no decomposition in urine under the same conditions, there was a slight decomposition in some urines. To shorten the time of distillation to a point where this slight decomposition might be disregarded, methyl alcohol was added to the urine. This lowered the boiling point of the mixture so that a more rapid ebullition took place and all of the ammonia was driven off by the end of fifteen minutes. Shaffer found the use of sodium carbonate preferable to the use of other alkalies, for with sodium carbonate there was no foaming to render the operation difficult, there was less decomposition than with lime and no greater than with magnesia, and the expulsion of the ammonia was as rapid when liberated from ammonium salts as from a solution of free ammonia.

The method of vacuum distillation as adapted by Shaffer was as follows: To 50 cc. test material were added an excess (15 or 20 grams) of sodium chloride, about 50 cc. of methyl alcohol, and 1 gram of sodium carbonate. The flask containing this mixture was surrounded by a water bath at 50°C. By the use of a water pump the pressure was reduced until the liquid boiled and the ammonia was driven off into two wash bottles containing N/10 sulphuric acid. At the end of fifteen minutes all ammonia had been given off. The acid was now titrated with N/10 sodium hydroxide, using alizarin red as an indicator. Shaffer stated that this indicator is not affected by the presence of ammonium salts and is very little sensitive to carbonic acid.

Sherman (1905) stated that the Boussingault-Shaffer method for the determination of ammonia in urine had been studied by Berg (1904) with regard to its applicability to the much smaller amounts of ammonia which occur in milk. The following method was used for the determination of ammonia in milk. To 50 cc. of the sample were added 50 cc. of methyl alcohol, 10 grams of sodium chloride, and 0.5 gram of sodium carbonate. Sherman states that the sodium carbonate liberates the ammonia from its salts, and the sodium chloride diminishes the dissociation of the carbonate and thus prevents or retards the cleavage of ammonia from the nitrogenous organic compounds in the milk. If the sodium chloride is omitted in the distillation, a larger amount of ammonia is obtained. "For convenience," Sherman says, "the ammonia thus easily split off from organic matter by dilute sodium carbonate at 55° to 60°C. is called cleavage ammonia."

Harris (1919–1920) used Shaffer's modification of Boussingault's vacuum distillation method for determining ammonia in various media and bacterial cultures. Harris, however, omitted the use of sodium chloride and kept the temperature of the water surrounding the distilling flask at 50°C.

In the present investigations, sodium chloride was omitted in the vacuum distillation of milk and the water bath kept at 50°C. As the resulting figures for the ammonia content of the 27 samples of milk (see table 1) appeared too large when compared with the figures of Kendall, Day and Walker (1922), a control

TABLE 1

Sterile milk

	SAMPLE								
	I	II	III	IV	V	VI	VII	VIII	IX
†Nitrogen as ammonia	11.2	39.2	11.2	15.79	18.92	15.79	54.32	26.60	11.2
†Amino nitrogen (Wolf and Harris		14.31		10.60	23.84	46.27	10.17	13.35	42.63
†Amino nitrogen (Denis and Minot)		2.33	1.88	2.55	3.07	3.12	1.80	2.03	2.79
‡Reducing substances	2.93	4.25	4.04	4.25	4.48	4.14	4.36	4.14	4.19
pH	6.6	6.6	6.6	6.5	6.6	6.6	6.6	6.5	6.6
§Total acidity (1:10 dilution)	2.2	1.2	1.6	1.2	1.6	1.8	1.2	1.6	1.6
§Volatile acids	1.0	1.0	1.0	0.6	1.0	1.0	0.7	1.0	1.0

	SAMPLE								
	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
†Nitrogen as ammonia	38.04	25.59	32.76	17.36	17.36	18.87	32.48	46.59	26.32
†Amino nitrogen (Wolf and Harris)	33.2	28.40	24.09	21.85	28.10	29.42	31.73	24.03	25.04
†Amino nitrogen (Denis and Minot)	4.09	2.4	2.37	2.59	2.61	2.54	2.32	2.40	3.49
‡Reducing substances	4.36	3.67	3.94	3.94	3.36	4.48		4.25	4.09
pH	6.6	6.8	6.4	6.4	6.5	6.4	6.6	6.6	6.4
§Total acidity (1:10 dilution)	1.2	1.6	1.6	2.0	1.6	1.8	1.8	1.8	2.0
§Volatile acids	1.0	1.0	1.0	1.0	1.0	1.4	1.4	1.6	1.8

	SAMPLE								
	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII
†Nitrogen as ammonia	23.52	11.2	38.64	23.52	29.68	32.48	20.72	26.60	26.60
†Amino nitrogen (Wolf and Harris)	21.29		20.39	20.27	31.23	22.30	17.56	45.58	30.60
†Amino nitrogen (Denis and Minot)	2.30	2.43	3.29	3.94	3.06	3.37	2.10	2.50	2.10
‡Reducing substances	3.89	4.14	4.19	5.13	5.47	5.29	3.55	3.99	4.25
pH	6.5	6.6	6.6	6.4	6.6	6.4	6.4	6.4	6.4
§Total acidity (1:10 dilution)	2.2	1.8	2.0	2.8	3.2	2.6	2.0	2.0	3.2
§Volatile acids	1.6	1.6	1.2	1.6	1.6	1.6	1.4	1.2	2.0

† Milligrams per 100 cc. of the medium.

‡ Calculated as percentage of lactose.

§ Cubic centimeters of N/10 KOH required to neutralize 100 cc. of the medium.

analysis was made with one sample of milk to which sodium chloride was added. The analysis duplicated with the previous analysis in which sodium chloride was omitted. It was therefore indicated that the large ammonia figures obtained were not due to the omission of sodium chloride but to some other factor. Sherman (1905) says that "both the ammonia existing as such in milk, and the cleavage ammonia increased rapidly under conditions which favor the decomposition of proteids by bacteria or molds." Richmond (1914) says that the presence of ammonia in milk may be due to bacterial action. It is evident, therefore, that the amounts of ammonia present in milk would vary greatly, depending upon the age of the milk and the bacteria present. The commercial milk used throughout this study was analyzed at least thirty hours after milking.

AMINO NITROGEN

In the determination of amino nitrogen in milk, the Van Slyke method was used. Two methods of preparing the amino solution were used as a means of placing a check on the technique of the operation of the Van Slyke apparatus. Following the method described by Harris (1919–1920), the residue left in the distilling flask after the distillation of ammonia was acidified with acetic acid and filtered. The filtrate was then made up to 50 cc. and 1 cc. of this amino solution was used for the determination of amino nitrogen in the Van Slyke micro-apparatus. Duplicate solutions were prepared according to Harris' method and duplicate determinations were made of each solution. The largest percentage error accepted from the same solution, or from the average of the determinations from the two solutions, was 10 per cent.

The second method used was that devised by Denis and Minot (1919) for the determination of amino nitrogen in milk. Twenty cubic centimeters of milk were placed in a 200-cc. volumetric flask. To the medium were added 40 cc. of N/100 acetic acid, 10 cc. of 5 per cent copper acetate solution, and 60 cc. of distilled water. The flask was placed in a boiling water bath

for thirty minutes and at the end of that time 1 cc. of a 15 per cent solution of potassium oxalate was added. The mixture was cooled, brought to volume, and filtered through a dry filter paper. To the filtrate was now added 0.5 gram of powdered potassium oxalate and the solution allowed to stand for at least an hour before filtering. Fifty cubic centimeters of the final filtrate were evaporated on a water bath to 1 to 2 cc. and this concentrated amino solution was used for the determination of amino nitrogen by means of the Van Slyke apparatus. Duplicate solutions were made according to this method and duplicate determinations made of each solution. As in the solutions prepared by Harris' method, the largest percentage error accepted was 10 per cent.

On comparison of the results obtained from the determination of amino nitrogen from solutions prepared according to the method of Harris with results obtained by the method of Denis and Minot, it was observed that the actual amount of amino nitrogen in a sample of milk was much larger when determined from a solution prepared by the former method than when prepared according to the latter method.

When determined from solutions prepared by Harris' method, the amino nitrogen of sterilized milk varied from 10.17 to 46.27 mgm. per 100 cc. of the medium. The amounts of amino nitrogen in sterile milk, as determined from solution prepared by the method of Denis and Minot, ranged from 1.8 to 4.09 mgm. per 100 cc. of the medium. In table 1 are given the amounts of amino nitrogen in milligrams per 100 cc. of the medium for the 27 samples of sterile milk analyzed.

Several possible sources of error may be pointed out in Harris' method. Folin and Denis (1912) found that certain nitrogenous substances, such as creatine, asparagine, and tyrosine, when added to blood, could not be recovered quantitatively after precipitation with methyl alcohol. Greenwald (1915) concluded that alcohol precipitates some nitrogenous non-protein constituents of the blood, of which 25 to 50 per cent represents amino acid nitrogen as determined by Van Slyke's method. Bock (1916) found that amino acids added to the blood could not be recovered quantitatively after the use of methyl alcohol as a protein precipitant.

A second source of error is indicated by Van Slyke (1912). He states that it is essential that all the ethyl alcohol added to the medium before the ammonia determination should be driven off, for it decomposes the nitrous acid in the deaminizing vessel of the Van Slyke apparatus with the formation of large volumes of gases, which can be removed with permanganate only with difficulty and by the use of perfectly fresh permanganate solution.

A third source of error lies in the hydrolysis of the protein of the milk with an alkali. It is therefore evident that, while the amount of nitrogen in milk might be decreased by precipitation with methyl alcohol, the amount might also be increased by hydrolysis of the proteins during the removal of ammonia and by incomplete removal of the alcohol during this process. That there was a constant increase in the amino nitrogen of sterile milk when the amino solution was prepared by the method of Harris is shown in table 1.

LACTOSE

In determining the lactose content of milk, the method used was that devised by Folin and Denis (1918) for the determination of lactose in milk. The percentage of lactose in the 27 samples of milk analyzed ranged from 2.93 to 5.29 per cent.

HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentration of sterile milk varied in the twenty-seven samples from 6.4 to 6.6 pH. A 1:10 dilution of milk was used in the determination of hydrogen-ion concentration. Clark (1921) states that a well-buffered solution may often be diluted without seriously altering the pH. "When dealing with complex solutions which are mixtures of very weakly dissociated acids and bases and their salts, and especially when the solution is already near neutrality, dilution has a very small effect on the pH. . . . . Differences which may be observed are quite as likely to be due to the changes in the protein and salt content."

TOTAL ACIDITY

The total acidity of sterilized milk was determined by titration of a 1:10 dilution with N/10 potassium hydroxide, using phenolphthalein as an indicator. From 1.2 to 3.2 cc. of N/10 potassium hydroxide were required to neutralize 100 cc. of the solution. This gave a total acidity of 12 to 32 cc. of the undiluted milk.

VOLATILE ACIDS

The presence of volatile acids in sterile milk was determined by the steam distillation method of Dyer (1916) as modified from that of Ducleaux (1900). One hundred cubic centimeters of milk were made acid to Congo red and steam distilled. The distillation was carried out at a uniform rate for each experiment and the same amount of distillate collected (500 cc.). Harris (1919–1920) stated that, except for acids of low volatility, such as formic acid, this method would yield from 90 to 95 per cent of the total acid present. The distillate was then titrated with N/10 potassium hydroxide, using phenolphthalein as an indicator. As the amounts of volatile acids in milk were very small, 1 cc. of pipettes were used for the titrations. Results were expressed as cubic centimeter of N/10 potassium hydroxide per 100 cc. of the medium.

In the identification of the volatile acids in milk Dyer's qualitative color tests were used. One hundred cubic centimeters of the distillate obtained from the steam distillation of the medium were neutralized with potassium hydroxide and concentrated to about 20 cc. Portions of this concentrated distillate were used for tests for the presence of formic, acetic, butyric, valeric, and caproic acid. The volatile content of the twenty-seven samples of milk analyzed ranged from an acidity requiring from 0.7 to 2.0 cc. of N/10 potassium hydroxide to neutralize 100 cc. of the medium. While these figures are much lower than those given by Wolf and Harris (1916–1917), they are closely comparable to the amount given by Bushnell (1922). The volatile acid content of milk may be expected to vary widely with the amount and kinds of bacteria present and with the age of the milk.

Acetic acid, butyric acid, and caproic acid were identified in the distillate of milk by means of Dyer's colorimetric tests.

A reduction of dilute solutions of potassium permanganate was observed in a number of instances. As this was one of the tests used by Rettger (1901) for the recognition of the volatile sulphide which he found to be given off when milk is heated above 85°C., a positive test could not be taken as proving the presence of formic acid in milk.

Meigs and March (1913) found a constituent of milk which formed a large part of the unknown alcohol soluble substances, and which gave a strong test for unoxidized sulphur. It seems possible that this substance is identical with that noted by Rettger.

A volatile sulphide which blackened lead acetate paper was found by the writer to be given off from all samples of acidified or unacidified milk when subjected to steam distillation. A heavy blackish deposit was formed within the Liebig condenser at the point of condensation of the vapor from the distilling milk. As this precipitate gave a positive test for mercury, it is suggested that the blackish substance may be a compound of mercury (volatilized from the steam generator) and the sulphide given off by the heated milk.

SUMMARY

The amino nitrogen content of the same sample of milk varied widely with the method of preparing the amino solution for use in the Van Slyke apparatus. Closely duplicating results were obtained from the same solution under the same conditions by the use of the Van Slyke micro-apparatus.

All samples of milk, when subjected to steam distillation, gave off a volatile sulphide.

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A CHEESE FACTORY TEST FOR CRITICAL DEGREES OF ACIDITY[1]

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Modern chemistry has provided a means for classifying indicators, which greatly facilitates the selection of suitable ones for any given purpose.

Colorimetric hydrogen ion determinations usually can not be made with milk because the casein present adsorbs the color. However, casein can be precipitated from milk by rennet, and the fresh whey is said to have the same hydrogen ion content as the milk. In the cheese factory, these facts find practical application. Cheese is commonly made by curdling milk with rennet, and the whey exuding from the curd carries with it lactic acid produced by the bacteria in the curd.

Swiss cheese makers usually begin their daily work with entirely sweet milk, and sometime during the process of manufacture lactic acid begins to be formed. The first appearance of 0.01 per cent of lactic acid in the whey, raising its acidity from about 0.10 to 0.11 per cent can be readily detected by titration of samples of whey taken from the cheese kettle at intervals, with tenth normal alkali and phenolphthalein as in Manns' test. The appearance of lactic acid in the whey can also be detected by the electrometric method, or more simply by adding a suitable indicator to whey samples, taken at fifteen minute intervals. Acid may first appear any time from one to five hours after the milk is in the kettle. Our experiments to be published later show the importance of acid development.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

Electrometric determination on whey from the Swiss cheese kettle, kindly made for us in the laboratory of the University of Wisconsin Soils department by Horace J. Harper, Ph.D., Associate Professor of Soils, Iowa State College, show what may be expected. A sudden change occurs in the direction of the curve of pH values, when lactic acid formation begins. For cheese factory purposes, the use of an indicator should detect the same change, closely enough.

As long as the whey is quite sweet, 1 drop (or 2 drops) of 0.01 per cent solution of brom-cresol-purple indicator added to 1 cc.

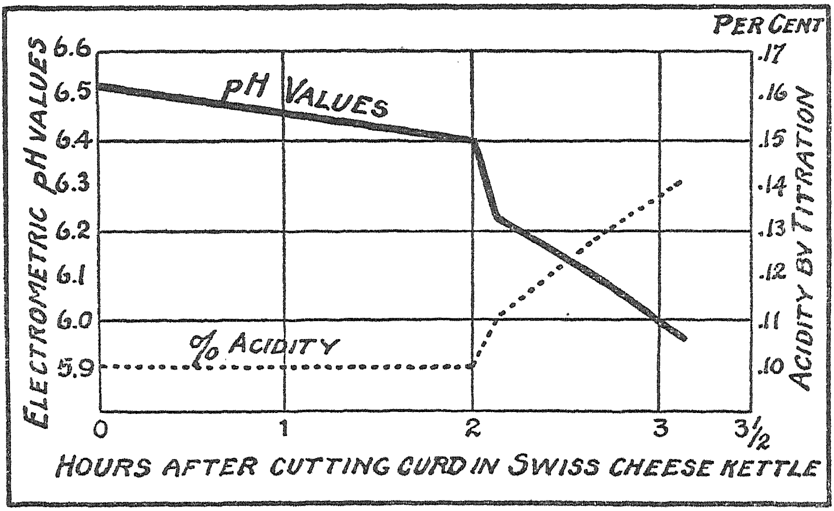

FIG. 1

of the well mixed whey in a test tube gives a distinct purple color, readily seen by comparison with another tube containing whey alone. When acid development begins in the kettle by bacterial action, the test shows gray, and with more acid, a yellow tint. Experience in this laboratory shows that this gray color change may be used as a guide by the cheese maker who has been waiting for acid development to begin in the kettle before he proceeds to the final steps in the Swiss cheese making process. The yellow color appears at about the point of acidity, 0.17 to 0.18 per cent, at which the American cheese maker draws the whey.

In a similar way, various indicators are being tried as means to detect the critical degrees of acidity required at important points in the manufacture of other kinds of cheese.

The test described above has been in use during several months past in cheese making experiments at the University of Wisconsin Dairy Department. The color test requires less time than the acidimeter test, and may become a distinct aid to the busy cheese maker in his daily work, although it can not replace the acidimeter in testing starters and other products of high and variable acidity.

THE INFLUENCE OF CERTAIN FACTORS ON THE HYDROGEN ION CONCENTRATION OF MILK. I

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This paper contains the results of an attempt to determine some of the causes influencing the reaction (acid or alkaline) of freshly drawn cow's milk. Various explanations have been offered to account for the acidity of fresh milk. Bordas (1) claimed the original acidity of cow's milk was due to free casein and not to lactic or citric acid or acid salts. Van Slyke and Bosworth (2) claim that the acidity of fresh milk is due to acid phosphates of the type MH_2PO_4. When titrated with NaOH insoluble $CaHPO_4$ is formed which hydrolyses to $Ca(OH)_2$ and H_3PO_4. The $Ca(OH)_2$ then unites with the di- and monocalcium phosphates to form insoluble tricalcium phosphate $Ca_3(PO_4)_2$ leaving the molecule of H_3PO_4 to react with more alkali.

Several factors are considered as influencing the acidity of milk, among which are the following: chemical composition (3), temperature of milk (4), pasteurization (5), feed (6, 7, 8, 10), age of cow (8), period of lactation (6, 8), and sickness (6).

There are three recognized ways of determining acidity in milk: titration with 0.1 N NaOH and phenolphthalein, Van Slyke's and Bosworth's modification of the above method and the determination of the hydrogen ion concentration.

Van Slyke and Baker (5) showed a definite relationship between the total (titratable) and actual acidity in milk. Further data is presented in this paper to show the relationship between the two ways of determining the acidity of milk.

EXPERIMENTAL

The following factors were studied in this investigation:

1. Effect of feeding acetic, butyric, lactic, and phosphoric acids.

2. Effect of changing from dry feed to pasture.

The cows used in this investigation were cows 43, 229 and 297 of the Purdue Dairy herd and were kindly loaned to the author for this work. Cow 43 was a Holstein, the other 2 were Jerseys. The cows had been giving milk for ten months at the time the experiments began and were receiving the following rations:

Grain mixture:
- 4 parts corn meal
- 2 parts wheat bran
- 2 parts ground oats
- 1 part oil meal (linseed)
- 1 part cotton seed meal

Daily ration

COW NUMBER	GRAIN	SILAGE	ALFALFA
	pounds	*pounds*	*pounds*
43	16.5	30	10
229	15.0	24	10
297	12.0	24	10

Cow 229 received $1\frac{1}{2}$ pounds of cottonseed meal per day in addition to the above during the first few weeks of the experiment. The cows all had automatic watering cups attached to their mangers, enabling them to have all the water desired. All cows were milked and fed three times per day until just before going on grass when nos. 229 and 297 were only milked twice per day.

Method of feeding the acids. The acids were fed by being thoroughly mixed with the grain in the evening at 7:00 p.m. just previous to the evening milking. No difficulty was experienced in getting the cows to eat the ration thus prepared until butyric and acetic acids were tried. After the first time, no difficulty was encountered with the butyric, but it was found necessary to dilute the concentrated acetic with an equal volume of water when the larger amounts were fed.

Careful notes were taken at all times on the behavior of the cows, the changes in weather, and the behavior of the apparatus.

Methods of sampling. Immediately after being milked, the milk was thoroughly stirred in the pail by means of a small dipper; the sample put in a small carbon dioxide flask and a few drops of toluol added to prevent bacterial action.

The samples were then cooled under the tap and kept cool until the determinations could be made. About an hour usually elapsed from the time the first sample was taken until the apparatus was set up and the first determination started. The time was recorded in all cases.

During the experiments the samples were taken from the morning milkings, unless otherwise specified, as in a few cases, where noon samples were used. The weight of the milk given per day for each cow is also included in an attempt to study what relation, if any, exists between the amount of milk given per day and the hydrogen ion concentration and the total acidity of the milk.

Control of temperature. The samples were kept cool by tap water until time to make the determinations, when they were warmed to about 21° to 22°C. by rotating in hot water, removing and shaking. It was possible by this method to keep the temperature variations for the titrations within a range of 3°C. The temperature of the samples for hydrogen ion determinations was governed by the temperature of the hydrogen in the tank, which seldom varied more than from 21° to 23°C. The temperature factor was also taken care of in the pH determinations by using the "difference of potential" factors worked out by Sorensen (9).

The apparatus. The apparatus used in this work consisted of a saturated calomel electrode, a modified Hildebrand hydrogen electrode, a storage battery wired to deliver 4 volts, a tank of hydrogen and two wash bottles containing alkaline pyrogallate and one containing water to wash the gas; a Leeds & Northrup Student's potentiometer, a resistance box and a galvanometer, were used to measure the potential.

The hydrogen was washed through the alkaline pyrogallate solution to remove the oxygen. The saturated calomel electrode was chosen because it is less influenced by a change in temperature and there is less contamination by diffusion from saturated KCl used in the bridge during the determinations, and, also less trouble from a difference of potential at the surface of contact of the liquids. The apparatus as used is sensitive to a change of 0.02 to 0.03 of a pH unit.

Method used to determine the hydrogen ion concentration. In making the pH determinations the sample was first brought to the proper temperature, as previously described, thoroughly shaken, about 20 cc. placed in a vessel (made by cutting a 100 cc. graduated cylinder off at the 30 cc. mark), the hydrogen electrode immersed in the milk and hydrogen bubbled through swiftly with occasional shakings for about five minutes, when the speed of bubbling was decreased somewhat. After about ten minutes had elapsed, readings were taken by immersing the tip of the calomel electrode in the milk and making the reading on the potentiometer when adjusted to show no current passing through the galvanometer.

After making the reading the calomel electrode was removed until a second reading was made two to three minutes later. Occasional readings were taken in this way until the sample and the electrodes had reached equilibrium. The final reading was taken with the gas bubbling through the milk at the rate of about 2 bubbles per second. Before making a final reading the resistance boxes were adjusted to be in equilibrium with the standard Weston cell. The following formula was used in making the calculations from the potentiometer readings:

$$\frac{\text{E.M.F. (observed)} - \text{E (calomel electrode)}}{0.0001983\ \text{T}} = \log\frac{1}{\text{H}} = \text{pH}$$

E.M.F. = the potentiometer reading in volts

E = the potential difference, referred to the normal hydrogen electrode of the saturated calomel electrode

T = absolute temperature

A sufficient number of the determinations were run in duplicate to insure the accuracy of the method, and the apparatus. The apparatus was also checked against standard buffer solutions from time to time as occasion seemed to demand.

The method used to determine the total acidity. The titration method previously described was used with the following modifications: only 50 cc. of milk were used for the titration, and the results doubled to represent values for 100 cc.

The effect on the acidity of milk of feeding certain acids to cows. The determinations were run on the milk for three days with the cows on a normal ration before starting to feed the acids. In the case of cow 297 the determinations were made again on normal feed after the lactic acid trials, while cow 43 was changed to a ration containing butyric acid. Cow 229 was used as a check and received no acid. Below are the results of the acid trials.

Discussion of the acid trials. From a study of the data in table 1 no striking changes seem to have been caused in either the H ion concentration or the total acidity by the presence of acids in the rations. Cow 297 received 85 per cent lactic acid as follows for a period of fourteen days; first four days 20 cc. per day; second four days 40 cc. per day; last six days 80 cc. per day; then followed a period of nine days when no acid was fed, then for a period of six days 50 cc. of 85 per cent phosphoric acid was added per day. No greater variation was noticed than is shown for cow 43 in table 1. Cow 229 was used as a check and received no acids in her feed. Her milk showed the same amount of variation as shown by the milk of the other cows. The small daily variations likely result from minor physiological disturbances.

Where the range is wide, a relation is shown to exist between a change in total acidity and a change in pH value; where the changes are small, no such relation holds true.

No apparent relation exists between amount of milk given per day and either the pH value or the total acidity.

From the foregoing data it would seem improbable that the reaction of cow's milk is influenced by the acidity of the ration.

TABLE 1

Reaction of milk when the acids were added to the ration: cow 43

DATE	POUNDS OF MILK PER DAY	H^+ ION CONCENTRATION AS pH	TEMPERATURE OF MILK FOR pH DETERMINATION	TOTAL ACIDITY cc. N/10 NaOH	TEMPERATURE OF MILK FOR TOTAL ACIDITY	AMOUNT AND KIND OF ACID FED PER DAY
1922			°*C.*		°*C.*	
April 11	36.2	6.52	20.0	23.00	20.0	None
April 12	37.7	No sample taken				None
April 13	38.4	6.58	21.0	25.40	23.0	None
April 14	39.3	6.53	22.0	24.90	24.0	20 cc. 85 per cent lactic
April 15	39.8	6.50	25.0	25.50	24.0	20 cc. 85 per cent lactic
April 16	39.7	6.55	22.0	25.05	23.0	20 cc. 85 per cent lactic
April 17	39.6	6.55	22.5	25.60	23.0	20 cc. 85 per cent lactic
April 18	37.9	6.54	20.0	25.70	22.0	40 cc. 85 per cent lactic
April 19	39.1	6.53	22.0	26.10	24.0	40 cc. 85 per cent lactic
April 20	40.0	6.53	21.5	25.09	22.0	None
April 21	38.4	6.53	21.0	25.75	23.0	None
April 22	40.4	6.50	24.0	26.15	24.0	None
April 23	39.5	6.54	20.0	26.95	21.0	40 cc. 60 per cent butyric
April 24	39.5	6.44	21.0	26.30	22.5	40 cc. 60 per cent butyric
April 25	39.9	6.43	22.0			40 cc. 60 per cent butyric
April 26	39.9	6.42	23.0	25.10	23.5	80 cc. 60 per cent butyric
April 27	41.9	6.51	21.0	25.20	23.0	100 cc. 60 per cent butyric
April 28	42.2	6.54	21.0	25.00	23.0	100 cc. 60 per cent butyric
April 29	38.9	6.61	20.5	23.80	23.0	None
April 30	40.5	6.49	21.0	24.80	22.5	None
May 1	41.6	6.50	21.5	24.30	23.0	None
May 2	41.6	6.56	22.5	23.90	23.5	None
May 3	40.8	6.48	24.0	24.00	25.0	None
May 4	42.6	6.46	23.0	24.10	25.0	None
May 5	42.9	6.56	21.5	24.00	23.0	None
May 6	40.9	6.56	21.5	23.40	22.3	None
May 7	No samples taken					50 cc. glacial acetic
May 8	41.6	6.50	20.0	23.30	21.0	50 cc. glacial acetic
May 9	43.2	6.45	22.0	23.70	23.0	50 cc. glacial acetic
May 10	41.4	6.48	22.5	23.00	24.0	50 cc. glacial acetic
May 11	39.8	6.56	22.0	23.70	23.5	None
May 12	37.5	6.50	23.5	21.60	24.0	None
May 13	33.6	6.44	22.0	24.52	23.0	None

Effect of changing from winter ration to summer ration. In table 2 data are presented to show the effect of changing from a winter ration including no pasture, but including silage, to a summer ration containing pasture instead of silage.

From the averages in the pH value little change can be detected. A small increase in acidity, as pH, is noted with cow 43, a similar decrease with cow 229; a larger decrease in acidity as pH, is however shown with cow 297. These changes, partly contradictory, are probably, but not necessarily, due to other causes than the change of ration.

Quite uniform decreases in total acidity are shown when the averages representing the winter ration are considered. This decrease holds in all cases. Further work is desirable here, in order to determine what change, if any, is shown by the solid constituents of milk when a grass ration is substituted for a winter ration. The above results also corroborate the statement made previously that a close relationship does not necessarily exist between the pH and the total acidity.

TABLE 2

Effect of dry feed compared to pasture on acidty of milk

COW NUMBER	DATE		POUNDS OF MILK PER DAY	H^+ ION CONCENTRATION AS pH	TOTAL ACIDITY, cc. N/10 NaOH	KIND OF RATION
	From	To				
	1922	*1922*				
43	April 28	May 2	42.0	6.54	24.83	Dry
43	May 23	May 27	34.9	6.51	22.31	Pasture
297	April 28	May 2	22.0	6.53	24.32	Dry
297	April 23	May 26	18.4	6.61	19.54	Pasture
229	April 11	April 15	26.75	6.63	24.99	Dry
229	May 23	May 27	17.1	6.65	21.55	Pasture

From the foregoing, it seems possible to influence the total acidity of milk by a change from a winter to a pasture ration, but not the pH value. More work is desirable along these lines.

CONCLUSION

Studies were made to determine the effect of feeding lactic, butyric, acetic and phosphoric acids in the H^+ ion concentration and total acidity of milk; to determine the changes, if any, produced when the cows were changed from a dry ration to a pasture ration. Little, if any, effect on the reaction of milk was noticed from feeding the above mentioned acids, either on the H^+ ion

concentration or total acidity. A marked decrease in total acidity was noted when the cows were changed from the winter ration to the summer ration, with practically no change in the pH value.

The author takes this occasion to thank Dr. Ernest Anderson, of the University of Nebraska, for very kindly suggesting the problem and preliminary methods, the Purdue Dairy Department for loaning the cows, Dr. P. N. Evans of the Chemistry Department of Purdue, for furnishing supplies, and Dr. Hoffer and Mr. John Trost for the use of their laboratory and apparatus.

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STUDIES IN THE GROWTH AND NUTRITION OF DAIRY CALVES

IX. THE ADDITION OF TOMATOES TO A MILK RATION

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In an effort to determine some of the fundamental principles underlying nutrition in general, and calf feeding in particular, a number of investigations have been under way for five years. In papers V and VI of this series, attention was given to the bulk supplied to young calves, though subsidiary problems also occurred. This paper simply includes further work on the influence of bulk on the value of the ration for a young growing calf, though at times other branches of the work have to be brought in.

RÉSUMÉ OF PREVIOUS WORK

All of the work dealing directly with the provision of bulk in the ration of the calf has been reviewed in the two previous reports, but attention may be drawn here to recent work which is applicable to some extent to this problem.

In papers V and VI of this series it was found that milk alone was not a good ration for calves but a ration of milk and grain was even poorer as the grains added so much more magnesium to the ration that the calcium used in the removal of the magnesium had actually to be withdrawn from the body stores. The introduction of alfalfa hay to the ration caused rapid improvement in the lime-magnesia ratio and so lead to a positive calcium balance. The bulk of the alfalfa hay was also of great value in bringing the digestion of the calves to normal as bulky feeds are necessary for the distension of the rumen and other parts of the digestive tract.

Since the compilation of the work by Eddy (64) on the distribution of the vitamines, it is easier to determine what influence the vitamines may have had in the work reported in papers V and VI.

From this it is apparent that the milk was supplying as much fat-soluble A and water-soluble B and considerably more water-soluble C than any of the grains used or than the alfalfa. Consequently the recovery of the calves when fed alfalfa hay can not be looked on as due to any additional amounts of water-soluble C provided. This means that only the calcium content of the alfalfa hay or its bulky nature could have been of value in improving the ration of the calves.

TABLE 65

Distribution of vitamines, from Eddy (64)

SOURCE	FAT-SOLUBLE A	WATER-SOLUBLE B	WATER-SOLUBLE C
Milk	xxx	xxx	xx
Alfalfa	xxx	xxx	?
Corn, yellow	x	xxx	?
Corn, white	0	xxx	?
Oats	x	xxx	0
Bran	0	x	0
Oil meal	xx	xxx	
Tomatoes, canned			xx

xxx indicates abundance, xx relatively large amounts, x present in small amounts, 0 absent.

On feeding grain and oat straw to goats for three months, Steenbock and Hart (67) found that the calcium balance remained negative. Then there were indications of approaching collapse on the part of the animals, such as lack of appetite and loss of interest in their surroundings. Fresh grass was then fed to the animals and they recovered.

Later Hart, Steenbock and Hoppert (65) continued this wo using dry and milking goats. When fresh green oats were fed the cacium balance was positive but on feeding oat straw, the calcium balance was negative. When the fresh green oats were dried in a well lighted and well ventilated attic but out of direct sunlight, they still retained some of the properties which aided in

calcium assimilation. It was also found that alfalfa hay kept up the calcium balance. Orange juice and cabbage had not this power so it can not be the antiscorbutic vitamine that aided calcium assimilation, but rather an antirichitic vitamine also found in cod-liver oil.

EXPERIMENTAL WORK

In the trial reported here 2 calves were used and although they were born some time apart, the records have been kept for each by thirty-day periods. Information concerning these animals is given in table 66. Calf 544 was twinned with a bull and was therefore presumed to be a free-martin, but a discussion of its sexual make-up will be taken up later. Calf 539 died at 180 days of age, being on a ration of milk alone, while calf 544 was killed

TABLE 66

Animal used

	CALF 539	CALF 544
Breed	Grade Jersey	Holstein
Sex	Male	Free-martin
Date of birth	November 3, 1920	December 26, 1920
Birth weight, *pounds*	55	65

after the completion of the fifteenth period. During the trial it had been fed milk, milk and canned tomatoes, and milk and alfalfa hay.

The feed consumption for each calf is presented by thirty-day periods. Calf 539 was fed milk alone and died at 180 days of age. Calf 544 was fed milk alone until the end of period VII when his appetite had decreased. An allowance of 1 pound, 3 ounces of canned tomatoes was then given for five periods but as this did not produce the desired improvement, alfalfa hay was fed at free-will until the trial was closed.

The live weights of the experimental calves were obtained at birth and at the end of each thirty-day period, by weighing on three consecutive days. The body measurements, height at withers, depth of chest, and width at hooks were obtained at the end of each period.

TABLE 67

Feed consumption by thirty-day periods

PERIOD NUMBER	CALF 544			COW 539
	Whole milk	Canned tomatoes	Alfalfa hay	Whole milk
	pounds	*pounds*	*pounds*	*pounds*
I	360			221
II	420			323
III	467			409
IV	624			495
V	765			645
VI	1181			675
VII	1125			
VIII	1125	36		
IX	1125	36		
X	1305	36		
XI	1310	36		
XII	1350	36		
XIII	1500		280	
XIV	1500		206	
XV	1500		285	

TABLE 68

Live weight and body measurements

AGE	AVERAGE FOR 40 HEIFERS				CALF 544				CALF 539			
	Weight	Height	Depth	Width	Weight	Height	Depth	Width	Weight	Height	Depth	Width
days	*lbs.*	*in.*	*in.*	*in.*	*lbs.*	*in.*	*in.*	*in.*	*lbs.*	*in.*	*in.*	*in.*
Birth	67				65				55			
30	90	28.7	11.4	6.7	99	32.7	14.8	7.8	72	31.6	13.7	8.0
60	120	30.7	12.8	7.5	150	33.3	15.1	8.6	91	32.6	14.1	8.0
90	163	32.9	14.2	8.5	183	33.9	15.6	9.2	123	33.3	14.2	8.0
120	211	34.8	15.4	9.4	251	34.5	16.0	9.4	165	35.1	15.3	8.9
150	262	36.4	16.7	10.2	301	36.9	17.2	10.3	237	36.2	16.1	9.5
180	314	38.4	17.9	11.2	390	39.0	18.2	11.5	250	37.0	16.5	9.7
210	366	39.4	18.7	11.8	437	40.3	19.1	12.3				
240	413	41.0	19.5	12.6	479	41.5	20.3	12.9				
270	452	42.0	20.3	13.2	481	43.0	21.3	13.3				
300	486	42.7	20.9	13.6	537	46.9	21.5	13.9				
330	521	43.5	21.5	14.2	554	47.6	21.7	14.0				
360	557	44.3	21.9	14.6	630	48.2	22.1	14.8				
390	592	44.9	22.4	15.1	721	49.4	22.7	15.4				
420	628	45.5	22.8	15.4	750	50.0	24.3	16.0				
450	659	45.9	23.2	15.7	795	50.8	25.7	16.0				

RESULTS SECURED

If the increase in live weight and body measurement be taken each third month and expressed as percentages of the first measurements taken, they can be compared with the averages for a group of 40 heifers raised normally and reported in paper III of this series. In making this comparison in table 69 it will be found that calf 539 shows a tendency throughout to be below normal in both live weight and body measurements. On the other hand, calf 544 always shows a higher rate of increase in live weight than is found in the normal group. However, this calf shows slow development in the body measurements until the alfalfa period is reached.

TABLE 69

Percentage increase in live weight and body measurements

AGE	AVERAGE FOR 40 HEIFERS				CALF 544				CALF 539			
	Weight	Height	Depth	Width	Weight	Height	Depth	Width	Weight	Height	Depth	Width
months	*lbs.*	*in.*	*in.*	*in.*	*lbs.*	*in.*	*in.*	*in.*	*lbs.*	*in.*	*in.*	*in.*
3	143	15	25	27	181	4	4	18	124	5	4	0
6	369	34	57	67	500	19	12	47	354	14	21	21
9	575	46	78	97	640	31	44	71				
12	731	54	92	118	869	47	50	90				
15	884	60	104	134	1123	55	74	105				

It is well to study the requirements of the calves and the supply of nutrients supplied to them as this gives an idea of how far their rations meet their needs for nutrients, and other factors concerning the rations can be considered later. The nutrients required by and supplied to the calves have been calculated from the work of Henry and Morrison (66). To simplify matters however, the excess or deficit of nutrients supplied during each thirty-day period has been calculated for each calf.

The calf 539 always had an oversupply of nutrients until the end of the fourth period. Then the supply of total dry matter dropped below normal and the supplies of digestible nutrients came down to near the actual requirements. In the next period

the deficit of dry matter was even greater and the supply of digestible crude protein and digestible carbohydrate equivalent was below that required by the calf. The animal was given all the milk it could handle and yet could not obtain sufficient nutrients from it and the calf died at 180 days of age.

In the case of the larger calf 544, there was a deficit in the dry matter supply from the first period. This deficit varied irregularly but at the end of period VII, it was greater than at the

TABLE 70

Percentage of nutrients in addition to requirements provided

PERIOD	CALF 544			CALF 539		
	Total dry matter	Digestible nutrients		Total dry matter	Digestible nutrients	
		Crude protein	Total		Crude protein	Total
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	−56	60	64	11	20	25
II	−62	36	44	26	38	7
III	−68	13	17	23	31	38
IV	−68	10	16	10	17	24
V	−69	4	11	−39	4	10
VI	−78	40	38	−48	−10	−5
VII	−68	36	10			
VIII	−85	32	2			
IX	−85	28	−2			
X	−84	47	3			
XI	−85	36	−2			
XII	−86	29	−4			
XIII	−22	130	54			
XIV	−42	90	33			
XV	−25	108	40			

beginning of the trial. At the start of the trial the calf consumed enough milk to provide a large excess of nutrients, but this excess decreased irregularly and reached a low point for total nutrients during period VII.

At the beginning of period VIII canned tomatoes were introduced into the ration and were fed with the milk for five periods, but during this time the deficit in dry matter consumed became greater. The amount of digestible protein consumed also de-

creased and where there had previously been an excess of total digestible nutrients a deficit became apparent.

At the beginning of period XIII the feeding of canned tomatoes was stopped and alfalfa hay was introduced into the ration. The deficit in dry matter was immediately largely reduced though it had not entirely disappeared when the calf was slaughtered after 90 days on this ration. The supply of total digestible nutrients was also immediately converted from a deficit into a surplus and the supply of protein was also greatly augmented.

BEHAVIOR OF THE CALVES

The calf 544 did not show any peculiarities in action until the fifth period when it started to chew the wooden walls of the pen and in period VI it showed signs of irritability or nervousness. These peculiarities continued to some extent until alfalfa was introduced into the ration. In period VII, just before the feeding of tomato juice was started, the calf was stiff in the joints and had a stilted gait. About this time the lack of middle in the calf also became apparent. The eyes also did not appear normal; they were watery and dull. When the tomato juice was introduced the calf picked up, walked better and its eyes appeared to be well, but within two months it was back in the old condition and the leg joints became puffy. This lasted until the introduction of alfalfa hay to the ration when the calf started to regain a normal appearance and looked in good shape when the trial ended.

In the case of calf 539 somewhat similar conditions prevailed. It started chewing wood in the second period and by the third period was extremely nervous even when touched. It continued in this condition until its death at the end of period VI.

POST-MORTEM EXAMINATION OF CALF 544

The calf 544 was killed at the end of the fifteenth period and an autopsy was conducted by Dr. E. A. Benbrook of the Department of Veterinary Pathology and Bacteriology, while Dr. H. S. Murphy of the Department of Veterinary Anatomy examined the sexual organs of the calf.

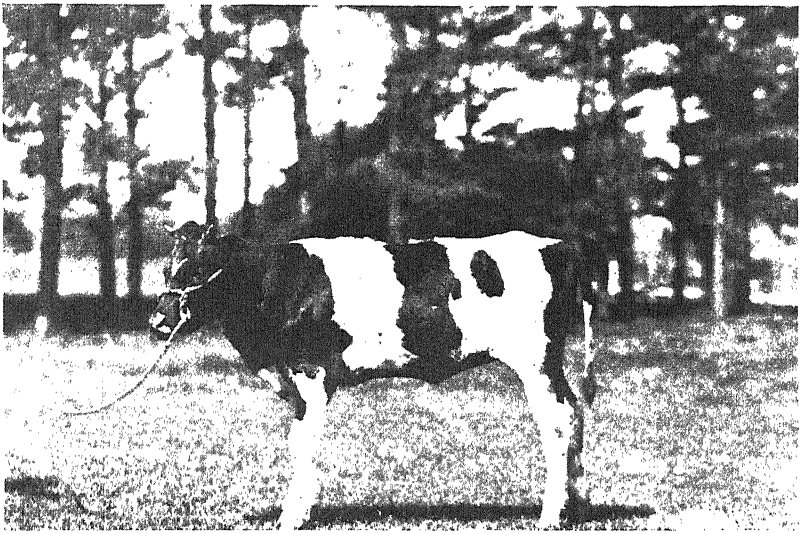

FIG. 1. CALF 544 JUST BEFORE CANNED TOMATOES WERE ADDED TO THE RATION

Note gaunt appearance. Age, 7 months

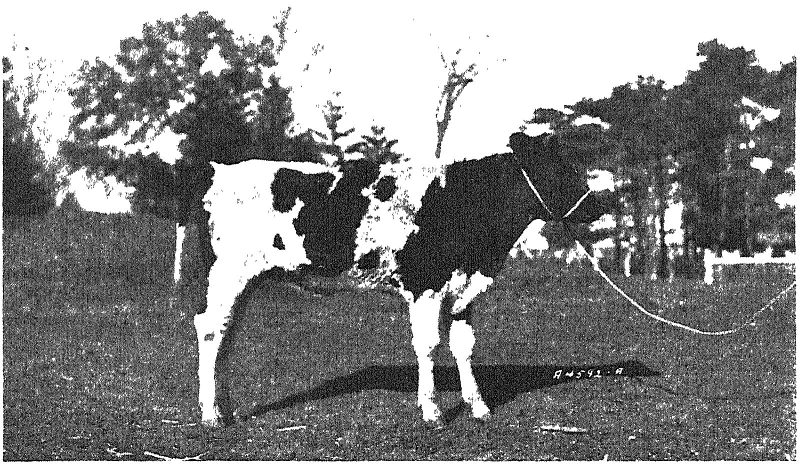

FIG. 2. CALF 544 AT TIME OF REMOVAL OF CANNED TOMATOES FROM AND ADDITION OF ALFALFA HAY TO RATION

Note poorer appearance and sunken eye. Age, 12 months

Fig. 3. Calf 544 after a Ration of Milk and Alfalfa Hay for 3 Months

Note bright appearance and development of middle. Age, 15 months

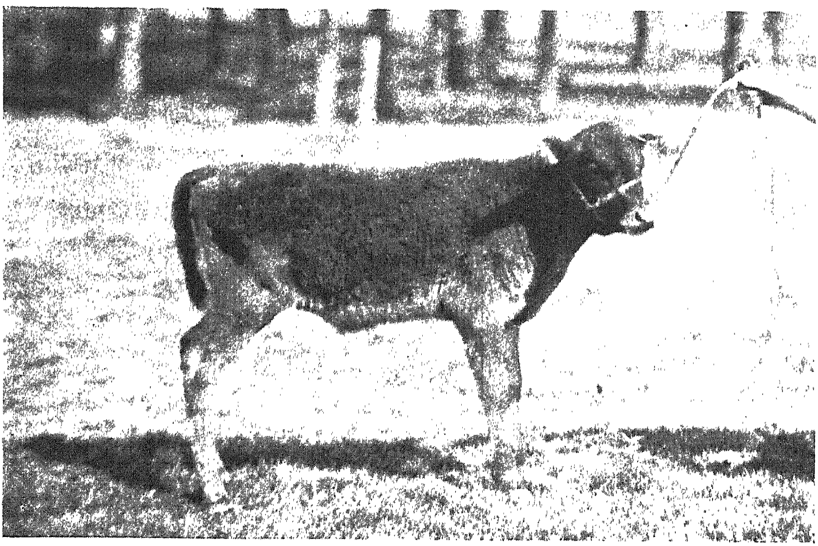

Fig. 4. Calf 539 on a Ration of Milk Alone

Age, 4 months

The animal was in fair condition and the abdomen was fairly well rounded yet flaccid. The condition of the skin was fair, though that in the region of the cheeks and jaws was dry, harsh, scaly and partly denuded of hair.

The stomach

The rumen, reticulum and omasum appeared to be normal in size, whereas, the abomasum was voluminous for an animal of this size. The contents of the stomach compartments were about normal, but in the rumen there were two hair balls, one about 4 inches in diameter with a brown smooth coat and the other about 1½ inches in diameter and uncoated. The reticulum contained no foreign bodies except a small amount of sand and cinders. The mucosa of the abomasum was slightly catarrhal.

The intestine

In both the large and small intestine the mucosa was somewhat catarrhal.

The glands

The liver was enlarged by about one-third, was pale yellowish-red in color, mottled and friable. The gall bladder contained a number of concretions and the mucosa was slightly catarrhal. The pancreas was slightly enlarged. The spleen was slightly enlarged. The mesenteric chain of lymph nodes were all somewhat enlarged and the cortex in most cases was slightly reddened. In some petechial hemorrhages were noted. The remainder of the lymphatic system was apparently normal so far as could be noted in the examination. The kidneys were slightly enlarged and pale. One kidney contained a retention cyst the size of a pea.

The thyroid glands were flattened and irregularly lobulated. The gland tissue was pale red in color and apparently interspersed with strands of connective tissue. The cut surface was shiny and rather sticky. The parathyroids were flattened and about the size of a pea. The thymus gland was large in its

extra-thoracic position, extending up to the larynx, and most of the gland lobules showed petechial hemorrhages. The intra-thoracic portion of the gland was small.

The skeletal tissues

The skeleton showed no noticeable changes. Cross sections of the ribs above and near the sternum and of the left tibia appeared to be normal.

The genital organs

This animal as mentioned earlier was twinned with a bull, but from anatomical and histological examination, Dr. H. S. Murphy of the Department of Veterinary Anatomy was of the belief that the animal was potentially a bull though sexual development had been arrested.

POST-MORTEM EXAMINATION OF CALF 539

This animal was examined shortly after death by Dr. E. A. Benbrook of the Department of Veterinary Pathology and Bacteriology.

The stomach

The rumen was about the size of the other three compartments of the stomach and it contained a grey-brown opaque liquid. An oval uncoated hair ball about 2 inches long and 1 inch in diameter was found in the rumen. The abomasal mucosa showed hemorrhages.

The intestine

The intestine showed no changes of importance.

The glands

The liver showed a cloudy swelling and a few subcapsular hemorrhages, while the gall bladder contained a small amount of yellow cloudy bile. All of the lymph nodes were enlarged and congested and those of the viscera showed hemorrhages. The kidneys showed cloudy swellings and were congested. The thymus gland was large and showed hemorrhages throughout.

The skeletal tissues

The femur and humerus showed a peripherally red marrow but the osseous tissue was apparently normal. The joints of the legs were enlarged.

Cause of death

The ultimate cause of death was hemorrhagic septicemia which had a good opportunity for development owing to the lowered condition of the calf.

DISCUSSION OF RESULTS

From the work presented in this and the two earlier papers in this series which deal with the problem of restricted rations for calves, it is apparent that milk is not suitable as the sole feed for calves when fed for any considerable time. From the amount of milk consumed by the calves on a ration of milk alone, it is apparent that they were being supplied with sufficient digestible nutrients for a time, but later on in the trials they could not consume enough milk to provide all the digestible nutrients they required.

At a much earlier stage in the trials the dry matter provided by the milk rations fell below the requirements of the calves and so it would appear that the calves were not being supplied with enough dry matter, especially in the form of bulky constituents.

It has been sometimes believed that milk was at times deficient in the vitamine water-soluble C, but from the bibliography presented and the results of the trial reported here on the addition of tomato juice to a milk ration, it becomes apparent that the vitamine fat soluble A, water-soluble B and water-soluble C, were present in sufficient amounts and did not cause the inefficiency of milk when used as the sole ration.

The one point of importance brought out by the results reported here is that the addition of a roughage such as alfalfa hay to a milk ration will bring the calves through in good shape even though they have gone off badly on a ration of milk alone. There may be three factors which render such a roughage valuable in the ration.

It is to begin with bulky and thus renders possible the proper distention and development of the rumen and other portions of the alimentary tract even at an early age. It has already been shown in a previous paper in this series that the addition to a milk ration of grains relatively rich in magnesium and poor in calcium will prevent the proper deposition of calcium in the skeletal tissues. Alfalfa hay is rich in calcium and so aids in maintaining the proper calcium balance.

As has been shown in the résumé of previous work certain green plants, such as oats, contain an antirachitic vitamine which aids calcium assimilation. By careful curing part of this vitamine is retained. The alfalfa hay used in this trial was of the second cutting and was put up rapidly without much exposure to direct sunlight, so there is a possibility that it contained some of this vitamine and was thus of greater value to the calf.

SUMMARY

From the work reported here it would appear that:

1. Milk can not be used as the sole ration for calves indefinitely.
2. A lack of the vitamines fat-soluble A, water-soluble B and water-soluble C, is not a cause of the inefficiency of milk.
3. A bulky roughage appears to be necessary for the calf.
4. A roughage such as good pea-green alfalfa may also provide some of the antirachitic vitamine and add to the lime supply of the calf.

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ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The eighteenth annual meeting of the American Dairy Science Association was held in the Court House at Syracuse, New York, October 8 and 9, 1923. The meeting was called to order by President Borland. After a few remarks he called for the report to the secretary-treasurer. The secretary-treasurer also read a financial statement and accountant's report of the condition of the books.

The Executive Committee submitted a budget of $550 to cover expenses of various offices and sections for the coming year. The budget was adopted.

J. A. Gamble reported for the Southern Division.

R. C. Fisher reported for the Eastern Division.

A report of the meeting to federate all dairy organizations was given by O. E. Reed.

J. A. Gamble reported as representative of the Association to the National Research Council. In connection with the National Research Council, H. P. Davis moved that the President in consultation with the Executive Committee and the American Society of Animal Production make such recommendations as they think will be in order. Seconded by Ragsdale. Passed.

C. H. Eckles reported as a member of a committee on relation with the National Research Council. He suggested that the previous motion be amended so that a representative from the Dairy Science Association to the Research Council be elected with the other officers for a period of three years.

Professor Hooper of Kentucky report on Southern Dairy Cattle Judging contest at Memphis.

C. W. Larson reported as a representative to a meeting of the American Biological Society. As a result of this report it was moved and passed that a committee be appointed to study the

matter of affiliation with the American Biological Society and report back to the Society in the future.

The following men reported briefly as officers of the different sections: Section I, O. E. Reed; Section II, H. A. Ruehe; Section III, E. M. Harmon; Section IV, George C. White.

Dr. Rogers briefly reported progress of the committee on Bacteriological Standards.

J. H. Frandsen, editor of the JOURNAL OF DAIRY SCIENCE, and Mr. E. H. Williams, representing Williams & Wilkins who publish the JOURNAL, spoke briefly regarding future plans for the JOURNAL.

A. J. Glover presented a resolution to be sent to the President of the United States requesting that the Dairy Division of the Department of Agriculture be changed to the Bureau of Dairying. This resolution was adopted.

The President appointed C. H. Eckles, C. W. Larson and R. S. Breed to recommend action of the American Dairy Science Association as to membership in the Union of American Biological Societies.

The President also appointed R. R. Graves, C. H. Eckles, C. W. Larson, J. A. Gamble and R. S. Breed to meet with the American Society of Animal Production and Society of Genetics and agree on matters to present to the National Research Council.

Brief reports were then given covering the work taken up in this year's sectional meetings.

Professor J. A. Gamble Secretary of the Production Section gave the following report:

The Production Section of the American Dairy Science Association was called to order by Chairman O. E. Reed at 9:30 p.m. October 9, 1923.

There were forty men present representing over twenty different "Dairy Husbandry Departments."

In the absence of the permanent Secretary, J. A. Gamble was asked to act in that capacity. Because of the absence of the President, O. E. Reed, in Europe during the past summer and the fact that the program was generally merged with the World's Dairy Congress, no official program of papers had been prepared.

A discussion relative to the standardization of research technique was lead by C. H. Eckles which brought out may interesting points. As a result of the discussion, the following committee was appointed to study the research report soon to be made available by the National Research Council and to bring in a report regarding the outline for research technique. The committee consisted of C. H. Eckles, H. P. Davis and E. P. Meigs.

W. W. Swett rendered a very able report for the Dairy Cattle Judging contest and also raised the point that the breeder of Brown Swiss wished to have their breed included in the judging contest. This matter was discussed at length by many of those present and it was finally voted to refer it to the departments eligible to send teams for further action at the meeting next year.

The matter of absences of judging teams from College was discussed at length and the matter finally laid on the table. H. P. Davis of Nebraska offered a resolution thanking the United States Department of Agriculture and through it, the United States Dairy Division, for the very great help and assistance rendered the American Dairy Science Association in the contest and other work.

Election of officers for Section I, as follows: J. J. Hooper of Kentucky, Chairman, J. A. Gamble of Maryland, Secretary.

Professor A. E. Dahlberg reported as follows from the Dairy Manufacturers' Section:

Dairy Manufacturers' Section of the American Dairy Science Association was called to order by Chairman Ruehe, Illinois October 8, 1923, in the Court House at Syracuse, New York. In the absence of Secretary Kiethley, P. S. Lucas was elected acting secretary.

Reporting the work of the Committee on Standards for Creamery Glassware, Professor Hunziker stated that there had existed an impression that standards held by the United States Bureau of Standards and those advocated by the American Dairy Science Association were at variance. That this impression is erroneous

was pointed out by the Committee at the last meeting at St. Paul. This impression gained credence because the standards adopted by the Bureau had not been given publicity. At the instance of the Committee these standards have been printed in practically the entire dairy press and copies may be obtained from the Committee Chairman, Professor Hunziker. The standards of the Bureau and Association are identical in every detail.

Reporting further, Professor Hunziker stated that a conference had been called of members of the Dairy Science Association and the United States Bureau of Chemists by Dr. Hortvet, for the purpose of formulating specifications for Standard Babcock Glassware for recommendation to the American Association Official Agricultural Chemists. Professors Hunziker, Bouska, and Dahlberg represented the Dairy Science Association. The specifications of the United States Bureau of Standards and the Dairy Science Association were adopted in full by Dr. Hortvet's committee. There is every reason to believe that these standards will be adopted by Dr. Hortvet's association. It is the wish of Professor Hunziker's committee that each state dairy department will familiarize itself with these specifications and exert its influence, when occasion arises to work toward their adoption in the respective states. The committee's report was adopted.

Commenting on the work of the Committee on Students Dairy Products Judging Contests, Professor Judkins, Massachusetts, was asked to give a report on such contests, especially as held at the Eastern States Exposition, said report to be made at the call meeting at 7.30 p.m., October 9. Chairman Ruehe urged adoption of a uniform set of rules and score cards by all colleges. The questioned Rule 14 was discussed but no action taken. Mr. S. C. Thompson stated that purchase of outside samples for scoring would obviate Rule 14 but that the expense entailed serious consideration. He urged the appointment of a committee to meet with the Dairy Show management to consider the revision of the contest rules.

It was moved by Professor Fisher, Connecticut, seconded and approved, that the old members present of the Committee on

Students' Products Judging Contest confer on rules with Professor Judkins and Secretary Skinner and report results at the call meeting October 9.

Dr. Roadhouse, California, spoke generally of the judging contest and suggested that trophies be awarded only at the banquet, that this sugestion be incorporated in the rules. Mr. S. C. Thompson reiterated this suggestion, and urged further that enthusiasm and interest be encouraged by so systemizing compilation of results that announcement of awards may be made earlier than heretofore.

To encourage training of judges of dairy products and general interest in the quality of dairy products, Dr. Roadhouse urged consideration of association members in the instruction of high school teachers for the training of their students in determining quality of these products.

The second meeting of the Dairy Manufacturers' Section of the American Dairy Science Association was called to order by Chairman Ruehe of Illinois. In the absence of Acting Secretary P. S. Lucas of Michigan, the Chairman appointed A. C. Dahlberg, New York, as acting secretary.

The Chairman then called for the report of the temporary committee on Dairy Products Judging Contests, of which Professor Judkins of Massachusetts was Chairman. Professor Judkins explained that he was Chairman of the Committee on Dairy Products Judging Contests for the Eastern Section of the American Dairy Science Association, and due to the fact that he had a report for this Committee, the Chairman had appointed him, together with other individuals to consider the revision of the Dairy Products Judging Congests for the American Dairy Science Association. The detailed report of the two committees, of which Professor Judkins was Chairman, was then read by him. A copy of this report is attached to the minutes of the meeting. After reading the entire report each point was then read and discussed.

Professor Sommers of Wisconsin suggested that the score card for milk should be revised by omitting the points now allotted to acidity and adding them to flavor. After a brief discussion of

this matter it was referred by Chairman Ruehe to the Permanent Committee on Dairy Products Scoring Contests.

1. The Committee recommended that the score cards for dairy products as formulated by the United States Department of Agriculture should be used in the next scoring contest, and if satisfactory used permanently hereafter.

2. The Committee recommended that in order to simplify the handling of the Students' Dairy Products Judging Contest, that the duties of the Committee on Students' Dairy Products Judging Contest include the question of score cards, grades and standards. Professor Sommers of Wisconsin commented that this was advisable due to the fact that the Permanent Committee on Score Cards should not be burdened with special score cards for students' judging contests. Professor Fisher deplored the present discrepancy between the Government and the American Dairy Science Association score cards on cheese and milk. He pointed out that the United States Department of Agriculture had developed a system of grades and standards which had been published in Service and Regulatory Announcement, United States Department of Agriculture 51 and Handbook for Use in Inspection of Whole Milk, American Cheese, Circular 157. The publication of these grades and standards based on government score cards gives the coaches a definite basis on which to train their students. He considered it therefore very desirable to try the government score cards for one year. Professor Fisher further urged the establishment of one standard score card for each of the Dairy Products and the need of coöperation towards this end with the United States Department of Agriculture.

3. The committee recommended that in view of the present unsettled condition with regard to the score card and grades and standards on ice cream that it should not be included in the regular contest next year; but that a separate judging be conducted by the teams at which the coaches and representative of the United States Department of Agriculture act of judges for the purpose of developing a satisfactory score card and commercial grades and standards.

Professor Olsen of Kansas stated that last year the American Dairy Science Association had changed the score card for milk so that the five points allotted to acidity were given to flavor. The Chairman was asked whether this had been adopted by the United States Department of Agriculture in their score cards. Professor Sommers of Wisconsin responded that he had corresponded with Professor Hammer of Iowa who recommended that the change be made, and that Professor Hammer stated that it had been referred to the committee on score cards, but had not been adopted. Professor Ruehe agreed that this was the understanding.

The Committee recommended that criticisms be given in judging only when a cut in score was made. The maximum cut for any wrong criticism should not exceed one point. Professor Guthrie of Cornell stated that when butter was slightly wavy in color, no cut in score should be made. A student should, however make a note in his criticisms that he observed this condition. Consequently this recommendation of the Committee was illogical. Professor Fisher of Connecticut responded that the judges at Springfield had considered this fact and had allowed for this discrepancy.

Chairman Ruehe then called upon C. E. Lee of Gridley Dairy Company, Milwaukee, for remarks upon this subject. Mr. Lee stated that he had lost considerable faith in the commercial scoring of butter due to the fact that the score was raised or lowered according to market conditions and market demands. He illustrated this by stating that a car of butter made from sweet cream was scored 89 on a falling market in Chicago, and that this same car of butter was later received in New York on a rising market and scored higher than extras. R. C. Potts of the United States Department of Agriculture responded with the statement that this idea of changeable scores was entirely wrong. He stated that it was to the advantage of the dealer in business to argue grades so far as possible to fit market conditions, but that it was entirely possible to score butter very consistently day after day. He suggested that two of the butter judges of the United States Department of Agriculture be placed upon test to deter-

mine how accurate they could judge. After further discussion upon this subject Chairman Ruehe suggested that it would be concluded in order that the subject matter at hand might be covered during the evening.

5. The Committee recommended that ten samples of each product to be judged be used in the scoring contest. The size of the tubs of butter to be used was briefly discussed and the matter was left to the discretion of the Superintendent of the Contest.

6. It was recommended that three samples of each of the products to be judged should be used as a standard and be available for inspection by students with the scores and criticisms as given by the judges. Considerable discussion followed concerning the order in which dairy products should be judged, and how soon after testing the standard scored products a student should be required to judge the particular products reported in the standard. The discussion also brought out the necessity of having nearly all grades of the product reported in each contest.

7. The Committee also recommended that the rules be published one year in advance of the contest.

8. It was also recommended that the coaches be invited to observe the grading of criticisms by the judges.

9. Rule 14 which has been under much discussion was changed so that no coach or any person aiding in training a team may act as an official or be in any way connected with the Dairy Products Exhibit of the National Dairy Exposition.

10. It was recommended that more interest in the contest be promoted by announcing the scores and trophies at a banquet.

The question was raised concerning the desirability of judging milk powders. Chairman Ruehe stated that it had been talked of but never adopted. The report of the committee was then adopted in full.

The committee on the appointment of officers for the ensuing year, of which Professor Roadhouse of California was Chairman, reported as follows: H. W. Gregory, Indiana, Chairman; R. C. Fisher, of Connecticut, Secretary. The Chairman was then instructed to cast a unanimous ballot for them.

Chairman Ruehe then suggested that the new Permanent Committee on Dairy Products Judging Contest and Score Cards for these contests should be appointed by the newly elected officers. Professor Fisher then recommended that due to the absence of the newly elected chairman, that the present chairman should appoint the committee. It was made in the form of a motion and carried. Chairman Ruehe raised the question of the number of members of the committee and their distribution. After considerable discussion the following committee was appointed: Professor H. A. Judkins, Massachusetts, Chairman; Mr. S. C. Thompson, United States Department of Agriculture; Professor M. Mortensen, Iowa; Professor T. D. Turnbow, California; and Professor H. W. Gregory, Indiana.

Mr. R. C. Potts of the United States Department of Agriculture stated that the judging of cream for butter making should be included in the contest due to the fact that the quality of the butter was so dependent upon that of the cream from which it was made. He further stated that the economic phases of dairy manufacturing were not getting due consideration, and recommended a special section for these problems. Professor Roadhouse of California stated that this work should be included in the present Dairy Manufacturers' Section. This belief was also shared by Chairman Ruehe. Mr. Potts made a motion that the Chairman appoint a committee on the economic phases of the dairy manufacturing to care for the needs of that particular angle of the dairy business. The motion was unanimously carried.

Report of Extension Section was given by Professor C. A. Hutton:

The meeting of the Extension Section, American Dairy Scienec Association was held in the Court House, Syracuse, New York, Monday evening, October 8, immediately following a meeting of the American Dairy Science Association. The meeting was called to order by the Chairman, Mr. E. M. Harmon of Missouri. In the absence of the Secretary, Mr. F. A. Buchanan of Virginia, was elected as temporary secretary.

Report of Bull Association Committee. Mr. J. G. Winkjer, Dairy Division, chairman of the committee, was absent and Mr.

L. A. Higgins of Mississippi, one of the other members of the committee, made a brief report stating that the committee has been working on a plan to shorten the constitution and by-laws of bull associations, but so far a definite form has not been worked out. He called attention to the splendid results which are now showing up in regard to the increased production of the daughters of bull association bulls compared with the dams of these daughters. Records have been secured in Pennsylvania, Kentucky and Tennessee. He emphasized the fact that at present it is difficult to secure records of daughters of bull association bulls due to the fact that there are only three sections in the United States having both cow testing associations and bull associations in operation.

Report of Calf Club Committee. Mr. E. J. Perry, New Jersey, chairman of the committee, made a brief report stating that the committee has been working on a program for calf club meetings and a uniform system of record keeping. A discussion of club work was entered into and a motion was made by Mr. C. S. Rhodes of Illinois, that the committee on calf clubs for next year be instructed to consider the relations of the dairy calf club work to that of dairy extension work and to make recommendations with regard to better coördination of the work of the club specialist and dairy specialist. The motion was carried.

Report of Committee on Cost of Production. Mr. Moffitt of Pennsylvania, made a brief report of the work of this committee stating that some work had been carried on in Pennsylvania in connection with cow testing association work.

Report of Committee on Milk Campaigns. A very complete and interesting written report was submitted by Miss Jessie M. Hoover, Milk Utilization Specialist, Dairy Division, Washington, D. C., chairman of the committee.

Report of Committee on Dairy Council Work. A very complete written report was submitted by Professor W. P. B. Lockwood of Massachusetts, chairman of the committee.

Report of Dairy Products Committee. An oral report was made by Mr. L. W. Morley, Pennsylvania, chairman of the committee, in which he outlined the work of the committee which has been

along the lines of improving the quality of butter and ice cream and some work has been done on over-run and marketing problems.

A nominating committee was appointed which made the following report: New officers elected were, for chairman, L. W. Morley, Pennsylvania; vice chairman, C. S. Rhodes, Illinois and secretary-treasurer, C. A. Hutton, Tennessee.

The meeting then adjourned to meet again Tuesday evening.

Tuesday evening October 9, the meeting was called to order by Mr. L. W. Morley, chairman, who called on Mr. Brownell of Michigan and Mr. Harmon of Missouri to open a discussion relative to district diary specialists. Mr. Brownell told of the work of the district dairy specialists in Michigan and outlined a plan by which they hope to have a specialist of this kind for each eight counties in the state. Mr. Harmon stated that they now have two district dairy specialists in Missouri and plan to put on at least three more in the near future. These specialists are assigned to certain counties or districts for specific work under the direction of the dairy project leader. The principal features of the work done by those specialists in Missouri is along the line of cow testing associations, bull associations and feed production. They are required to report at headquarters once each month for conferences. All the salary and expenses are paid from State Extension funds and the work is confined to counties not having county agents.

Mr. Moffitt of Pennsylvania was asked to explain the work in regard to the cost of milk production which has been carried on in Pennsylvania. He stated that considerable work had been done in connection with the cow testing associations and that the figures had been used by the Philadelphia milk dealers and dairymen in the fixing of prices. He emphasized the importance of this kind of work and suggested that it adds a new interest to cow testing association work and makes these associations more far-reaching.

The work is done entirely by the cow testing association testers. Record forms are furnished by the extension service of the state. Mr. J. N. McClain of the Dairy Division, Washington, D. C.,

suggested that cost accounting was really investigational work rather than extension or demonstration work. Mr. Moffitt explained that the results were similar to cow testing association work but were more far-reaching and valuable since they included more items of expense and were really more accurate in his respect than cow testing association records.

Mr. Harmon moved that a committee on cost accounting be appointed and instructed to report on methods for this work and forms to be used. The motion was carried.

Plans for the year's work were then outlined by the chairman of the various committees after which the section adjourned to meet with the general session of the American Dairy Science Association.

Personnel of Committees in Dairy Extension Group

Chairman, L. W. Morley, Penn. State College

Secretary-Treasurer, C. A. Hutton, Knoxville, Tenn.

1923–1924

Dairy Products

J. D. Brew.......Ithaca, N. Y.
A. W. Rudwick.......Ames, Iowa
G. N. Toby.......Knoxville, Tenn.
S. C. Thompson.......Dairy Division, Washington, D. C.

Feeding

E. B. Fitts.......State College, Pa.
C. B. Finley.......Ames, Iowa
W. J. Keegan.......Clemson College, S. C.
A. R. Merrill.......Storrs, Conn.
H. A. Harper.......Ithaca, N. Y.

Milk Campaigns

Miss Jessee Hoover.......Dairy Division, Washington, D. C.
W. B. Lockwood.......Amherst, Mass.
Miss Gladys Stillman.......Madison, Wis.
Miss Dorothy Bulkley.......Storrs, Conn.
Miss Mary E. Thomas.......Baton Rouge, La.

Bull Associations

L. A. Higgins.......Starkville, Mass.
C. G. Cushman.......Clemson College, S. C.
M. J. Regan.......Columbia, Mo.
Ivan McKillip.......Columbus, Ohio.
A. R. Merril.......Storrs, Conn.

Calf Clubs

E. J. Perry	New Brunswick, N. J.
E. M. Harmon	Columbia, Mo.
F. A. Buchanon	West Virginia
R. H. Olmstead	State College, Pa.
C. A. Hutton	Knoxville, Tenn.

Cow Testing Associations

G. L. Tailby	Ithaca, N. Y.
A. J. Cramer	Madison, Wis.
E. A. Hanson	St. Paul, Minn.
C. R. Gearhart	State College, Pa.
A. C. Baltzer	East Lansing, Mich.
J. C. McDowell (advisory)	Dairy Division, Washington, D.C.

Professor G. C. White gave the following report for Section IV.

The section held a two hour session on the evening of October 9 with twenty states represented. Other unofficial delegates from these states and the United States Department of Agriculture were present. Mr. Cummings of the American Guernsey Cattle Club was also present.

Two foreign delegates, Atsushi Mujawaki, M.S., of the Department of Dairy and Meat Technology, Hokkaido Imperial University, Japan and A. Poole Wilson, Dairy Inspector, Department of Agricultural and Technical Instruction for Ireland were called upon and told briefly of cow testing and record making in those countries.

The Breed Relations Committee was called upon for its report. The following comprise this committee: G. C. White, Chairman, Connecticut, J. B. Fitch, Kansas, C. E. Wylie, Tennessee, W. M. Regan, California, W. W. Yapp, Illinois, A. A. Borland, Pennsylvania, and H. N. Colman, Oregon. The committee met two days previous (Colman absent) and acted upon several matters. Dr. Eckles, Professor Harris, Mr. Musser of the American Guernsey Cattle Club, Mr. Baker of the American Jersey Cattle Club, Mr. Inman of the Brown-Swiss Breeders' Association, and Mr. Gardner of the Holstein-Friesian Association of America were also present.

The following matters presented by the committee chairman were duly considered and adopted by the Official Testing Section.

1. Cows owned by state colleges admitted to Advanced Registry without fees. This idea was adopted by resolution at the 1922 meetings. Professor Wylie handled this and he was successful in obtaining the removal of Advanced Registry fees with associations where the requirement obtained.

2. Uniform guarantees to the colleges against delinquent testing accounts. Professor Regan has had this matter under consideration since August. The Breed Relations Committee instructed Professors Regan and Harris to prepare a resolution as a basis for negotiation with the cattle clubs. This resolution was read and adopted by special vote by the Official Testing Section.

3. Uniform drug and feed rules for cows under test. As is the case with other important matters, each club has its own particular ideas about what the breeder can or cannot do. This confuses the supervisor. Professor Yapp has had the matter under consideration only since August. The Committee recommended that he be given more time to work this out by negotiating with the Cattle Clubs.

4. Uniform two day report blanks have been in use since April 1 with general satisfaction. Improvements will be made at successive reprintings as rapidly as possible. The chief difficulty at present is the size of the blank. A larger blank would make it possible to correct most of the present objections.

The chairman is working upon a uniform preliminary report blank. At present it is not possible to get all of the things wanted by different clubs on one card. It is hoped that a blank will be ready by January 1, 1924.

5. Preliminary dry milking. Very little new data has come to light since it was thoroughly studied by the Committee in 1922. The Committee gave consideration to the check method suggested by Mr. Baker of the American Jersey Cattle Club. It still feels that the dry milking is necessary to set a time for the beginning of the test period as well as to guard against attempt at fraud. Mr. Baker's method can well supplement this and it is hoped that some such method will be employed by other Associations. The representatives from other clubs spoke very strongly against dropping the preliminary milking. The space in the uniform

two-day report blank for the milk and test of the preliminary milking very greatly strengthens the official test. All but about ten states are now enforcing the preliminary dry milking on all breeds. The committee recommended that the question be referred to the Investigation Committee of the Official Testing Section.

6. One versus the two day test. In 1922 the American Jersey Cattle Club officials stated that they were thinking of accepting the one day test. They were requested to give the Committee a chance to study the problem. Professor Colman of Oregon was able to do some work along this line but as yet it is too scant to be conclusive. The Breed Relations Committee recommended that this be referred to the Investigation Committee.

7. The Committee, since the last meeting, requested the several Breed Associations to print the uniform rules of the American Dairy Science Association, but some of them did not wish to because they were not being enforced by all States.

8. The nomination committee (Regan, Hooper and Ragsdale) presented the names of the officers for re-election. The rules were suspended and the recommendation prevailed, R. T. Harris, President, H. N. Colman, Vice-President, and G. C. White, Secretary, being re-elected.

9. New Breed Relations Committee appointed: W. M. Regan, California; J. B. Fitch, Kansas; M. H. Campbell, Illinois; C. E. Wylie, Tennessee; A. A. Borland, Pennsylvania; J. R. Dice, North Dakota; G. C. White, Chairman, Connecticut.

10. New Investigation Committee appointed: W. W. Yapp, Illinois, Chairman; J. B. Fitch, Kansas; A. A. Borland, Pennsylvania; J. J. Hooper, Kentucky.

Report of first meeting with the National Research Council presented by J. A. Gamble:

"It was my pleasure and privilege as your delegate, to attend the annual meeting of the National Research Council on April 22, 1923, in the Research Council, 1701 Massachusetts Avenue, N. W., Washington, D. C.

The meeting was called to order at 10:15 by Dr. Lillie, President. There were twenty-five official delegates present together with six others present by invitation. The Societies represented were Botanical Society of America, American Genetic Association, American Society of Zoologists, American Society for Horticultural Science, American Society of Animal Production and the American Dairy Science Association, American Society of Agronomy, Society of American Bacteriologists, Botanical Society of America, American Association of Economic Entomology, Ecological Society of America and Society of American Foresters.

It is apparent that all of the National Associations interested in biology and agriculture are represented in this Division of the National Research Council.

One could not help but appreciate the splendid opportunities offered the different groups by the National Research Council to discuss common problems. It is apparent also that the Council offers a means for the development of projects which could not otherwise be undertaken.

The matters discussed at the meeting related to the appointment of officers and committees, reports from the representatives of different Societies listed and the admittance to the National Research Council of the American Dairy Science Association and the American Association of Animal Production.

The application of the American Dairy Science Association was the first to be acted upon and the acceptance into the National Research Council formally passed. This was followed by the acceptance of the American Association of Animal Production.

The Committee on Food and Nutrition which was ordered continued: L. B. Mendel of Yale, Chairman; H. C. Sherman, Columbia University; C. L. Alsberg, C. F. Langworthy, Graham Lusk, E. V. McCullum, L. B. Mendel, J. R. Murlin, Alonzo E. Taylor, Miss Ruth Wheeler.

Subcommittee on Animal Nutrition as follows: E. B. Forbes, C. H. Eckles, H. S. Grinley, F. B. Morrison, C. R. Moulton, R. A. Pearson, to which were added F. G. King and L. J. Cole.

The question was raised by your representative as to the field which the activities of this committee were supposed to cover.

Suggesting that there were certain phases of animal production which were not included in the scope of the Food and Nutrition Committees. It was accordingly

Moved: That the Division invite the American Dairy Science Association and the American Society of Animal Production to make recommendations to the Division with regards to such Committees as they desire to have appointed.

In conference with E. W. Sheets, delegate of the American Society for Animal Production, it was suggested that a joint report be prepared by both societies and prepared as one report to the Division of Geology and Agriculture of the National Research Council.

It seemed to us that there should be subcommittees on Genetics and on Animal and Plant Breeding.

Following the suggestions made at the Syracuse meeting by your representative, President A. A. Borland appointed the following as a committee for advice regarding committees and personnel to report the American Dairy Science Association in the National Research Council: R. R. Graves, Chairman; C. H. Eckles, R. S. Breed and J. A. Gamble."

The annual report of the Eastern Division for the year 1922–23 was given by Professor R. C. Fisher.

The second annual meeting of the Eastern Division of the American Dairy Science Association was held in connection with the Eastern States Exposition at Springfield, Massachusetts, September 19, 1923.

The following is a brief summary of the program presented:

1. Get-together luncheon and results of judges contests:

Ten states were represented at a get-together luncheon, coaches, students, superintendents and judges of the dairy-cattle and dairy products judging contests were invited. Results of the dairy products judging contests in which six teams competed were announced by the superintendent of the contest, Mr. Wm. White of the Dairy Division, United States Department of Agriculture. This contest was inaugurated two years ago, largely

through the efforts of the Eastern Division. Four products, milk, butter, cheese and ice cream are judged. A beautiful trophy of bronze, symbolizing the farm dairy products and the gifts of the dairy industry to mankind was donated by the eastern dairy interests and goes to the running team each year. The results of the contests are briefly as follows:

Dairy products: New Hampshire, Connecticut, Cornell, Maryland, Pennsylvania, Massachusetts.

Dairy cattle: Connecticut, Maryland, Cornell, Maine, Massachusetts, Pennsylvania, New Hampshire, Syracuse.

2. Report of Rules Committee on Student's Judging Contest by H. F. Judkins:

Professor Judkins presented the report of the Rules Committee. This report was based on a discussion at a meeting held the previous evening, at which were present five members of the United States Dairy Division and five coaches of the judging teams. At this gathering it was the concensus of opinion that Rule 14 of the National Dairy Products Rules was non-essential and should be abolished and that the following should be adopted in its place; "The samples of all products used in the contest must be selected from the market and from the National Dairy Show Exhibit."

Considerable time was given over to the discussion of this point at the general session and the following was adopted as expressing the recommendation of the Eastern Division:

> It is the sense of this meeting that it would be entirely proper and within the power of the officers of the American Dairy Science Association to request the National Dairy Association to place the Dairy Products Judging Contest back on the program of the National Dairy Show this year. It being understood that the products for this contest will be obtained from the market and not from the exhibits of the National Dairy Show.

As our national president, Professor Borland was present at the meeting a copy of the resolution was at once presented to him so he might take such action as he deemed necessary.

3. Dairy Extension Problems:

J. D. Brew, New York, Chairman. Professor Brew presented a very interesting and instructive paper on Dairy Extension

Problems. The high points brought out in the paper and the discussion which followed were:

(1) That the majority of extension departments were handicapped by the lack of funds.

(2) That there are certain types of problems or research work that cannot be carried out in the field; that, therefore, there is urgent need for closer coöperation between the extension worker and the experiment station worker.

(3) That there is need for more coöperation between the workers of the different states.

4. Interstate Coöperative Dairy Problems by G. C. White and Dr. E. S. Guthrie:

Professor White outlined the coöperative plan of exchanging sires between the Massachusetts Agricultural College, the New Hampshire State College and the Connecticut Agricultural College.

He further cited the possible coöperation study that might be carried on as to what objects should the College Dairy Herd be maintained.

Dr. Guthrie pointed out the good that may be derived from closer coöperation between states on problems that are common to each. He cited illustration of coöperative work. (1) Dr. Ellenberger of Vermont and Dr. Guthrie of New York (2), Judkins of Massachusetts and Fisher of Connecticut.

5. Courses of Study:

Professor White, in absence of Dr. H. B. Ellenberger, presented the report of this Committee:

The high points are as follows:

(1) There necessarily is and should be a great deal of variation in the Dairy curriculum of different institutions.

(2) Students should receive thorough grounding in the fundamental sciences related to the Dairy Industry.

(3) That practical work and experience may best be gained outside of College.

(4) That it would be desirable to require at least 3 months actual experience in Dairy Plant work for those majoring in Dairy Production.

6. Relationship of Colleges to Commercial Dairy Interests by R. C. Fisher:

High Points of the discussion:

Committees of the Eastern Division met with committees from the Commercial Dairy interests to study:

(1) Type of Courses best meeting needs of the industry.

(2) Research problems that need working out, and how such work may best be carried on.

(3) Placement of our students both for permanent and temporary positions.

7. Election of Officers:

The following officers were elected for the ensuing year: Chairman, R. C. Fisher; Vice-Chairman, A. A. Borland; Secretary-Treasurer, R. W. Smith, Jr.

The report of the annual meeting of the Southern Division was given by Professor J. A. Gamble. The report of this meeting was printed in the Journal of Dairy Science, vol. vi, no. 4, July, 1923, page 373.

Following these reports the eighteenth annual meeting of the American Dairy Science Association adjourned.

The December ballot showed the re-election of the following officers for 1924: President, A. A. Borland, State College, Pennsylvania; Vice-President, O. E. Reed, East Lansing, Michigan; Secretary-Treasurer, J. B. Fitch, Manhattan, Kansas; Editor, J. H. Frandsen, Lincoln, Nebraska.

Report of Rules Committee for Students Dairy Products Judging Contest at Eastern States Exposition and special committee designated by chairman Ruehe to report for the Students Dairy Products Judging Contest Committee of the American Dairy Science Association:

The following report is based on a discussion at a meeting held at Hotel Bridgeway, Springfield, Massachusetts, Monday evening, September 7, at which the following were present: Campbell, Smith, White, Fryhofer, Williams, of the United States Department of Agriculture; and Harvey, Maryland; Combs, Penn-

sylvania; Judkins, Massachusetts; Fisher, Connecticut; Guthrie, New York; Lockwood, Massachusetts.

At a meeting at the Yates Hotel, Syracuse, New York, October 9, the following were present: Guthrie, Thompson, Harvey, Gamble, Potts, Kelly, Fisher, Judkins.

The following points were discussed:

1. Relation of judging contest and score card committee of the American Dairy Science Association to those in charge of formulating government score cards. The discussion brought out the points (1) that established government score cards might be modified to suit the American Dairy Science Association. (2) The new cheese score card printed in the "Handbook for Use in Inspection Law" was modeled after Wisconsin Standards, where a large percentage of our cheese is made. (3) The United States Department of Agriculture should be the clearing house calling the attention of the American Dairy Science Association score cards committee to proposed changes in cards and getting their action thereon.

It was moved and carried that the final score card should be the result of close coöperation between the American Dairy Science Association and other parties involved in the preparation of score cards.

It was pointed out that it did not matter as much what the score card was as that there should be uniformity in United States Department of Agriculture cards and those used in the judging contests.

It was also pointed out that once a score card becomes established as has a score card for butter it should be let alone unless there is some extraordinary reason for change.

2. The cheese score card. It was the sense of the meeting that the attention of the American Dairy Science Association score card committee be called to the great discrepancy between the United States Department of Agriculture and the American Dairy Science Association cards with a view to neutralizing the present difficult situation.

3. The use of the government score card for milk. It is recommended that the American Dairy Science Association score card

committee give thorough consideration to the difference in score allowed for flavor on the United States Department of Agriculture and the American Dairy Science Association score cards so as to bring uniformity about if possible.

After considering these three points at our meeting this morning it was recommended that all score cards for dairy products followed by the United States Department of Agriculture be adopted by our association for the period of one year, as a trial, with the understanding that if it is satisfactory that they will be permanently employed in our student judging contests.

It is further recommended that in order to simplify the handling of the students dairy products judging contests, that the duties of the committee on students dairy products judging contest include the question of score cards and commercial grades and standards, leaving the matter of legal standards to a separate committee.

4. The inclusion of ice cream in the national contest and score card for same.

Recommended that ice cream be included in the national contest using the American Dairy Science Association score card for want of anything better, confining the scoring to flavor, body and texture, color and package, and that the American Dairy Science Association score card committee work out a standard system of cuts on these items.

The committee this morning recommended that in view of the present unsettled condition with regard to the score card and grades and standards on ice cream, that it be not included in the regular contest next year; we suggest, however, that a separate judging be conducted by the teams, at which the coaches and a representative of the United States Department of Agriculture act as judges for the purpose of developing a satisfactory score card and commercial grades and standards.

5. The method of scoring criticisms.

Recommended that when a cut is made on any item the criticism be stated whether good or bad. When no cut is made no criticism is necessary. That in grading criticisms the maximum cut for each item shall be one point. It was pointed out that this

greatly simplified grading criticisms over the rule providing that not more than 5 points be scored off on any one sample.

6. The number of samples of each product to be included in the contest.

Recommended that five samples be used instead of ten, especially if ice cream is used in the contest. It was pointed out that a sufficient range of defects could be obtained in five carefully selected samples.

In view of the fact that ice cream be not included, recommended that ten samples of each product be used.

7. Number of representative samples to be placed before students before contest begins; should not judge's scores and criticisms be placed on the samples.

Recommended that two samples with score and criticism be placed before students.

Recommended that three representative samples of each product showing good, medium and poor quality be set out with the judges' scores and criticisms attached. The contestants shall have access to these samples, five minutes for each product, just previous to the scoring of the contest samples.

8. The importance of a commercial and United States Department Agriculture judge on products when both can be obtained.

It was felt that both a commercial and United States Department of Agriculture judge should be used on all products possible.

9. Voted in so far as past experience has proved that the rules of the contest have come out too late to be of greatest service in preparing teams, that rules be published at least one year in advance, i.e., new rules adopted at the annual meeting of the American Dairy Science Association would not become effective until a year later. Rules for the next contest shall be this year's rules with the changes authorized at this meeting.

10. Uniformity in work of official judges.

The discrepancy between the standard of the cheese judges at the Eastern States and National contests in 1922 was pointed out and it was voted that the rule providing "no judge can serve for more than 2 consecutive years and not more than one judge can hold over from one year to another" be abolished as it defeats the purpose of obtaining uniformity in standards of judging.

11. The use of standard terms by judges.

Recommended that official judges, in making criticisms stick rigidly to the terminology laid down in the prepared system of scoring cuts that have been uniformly used by the coaches in training teams.

12. Should coaches help in grading criticisms.

Recommended that it is very important that it be stated in the rules that coaches aid in grading criticisms.

Grading of criticisms shall be done by the official judge but coaches are invited to be present.

13. Action on Rule 14 providing "no member of the teaching, extension or experimental staff of a college entering a team may act as an official or be in any way connected with the dairy products of the National Dairy Show" be abolished and the following adopted in its place: "the samples of all products used in the contest must be selected from the market and not from the National Dairy Show Exhibit."

Recommended that Rule 14 be modified to read as follows: "No coach or any person aiding in training a team may act as an official or be in any way connected with the Dairy-products exhibit of the National Dairy Show."

14. Importance of the Contest.

Recommended that an attempt be made to emphasize more strongly the importance of the contest and raise its prestige.

READING THE FAT IN CREAM TESTS

GEORGE SPITZER AND W. F. EPPLE

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Received for publication November 12, 1923

Owing to the recent criticisms and misunderstanding concerning the manner of reading the test in the Babcock method for cream testing, we deemed it advisable to give a brief review and offer some additional data. It is not our intention to propose any modifications, but rather to substantiate and verify the accuracy of cream testing as compared with the official Roes-Gottlieb method for fat determinations.

The question of inconsistant results obtained by the Babcock method for cream testing when compared with the gravimetric method, has been the cause of numerous publications proposing modifications which should give most concordant results.

In 1903–1904, Ed. H. Webster, Bureau of Animal Industry, Bulletin No. 58, made a study of the testing of cream. The subject matter of this investigation which concerns this article, is his work and results on the value or per cent of butter fat included in the meniscus in cream testing.

Webster in his investigation found the value of the meniscus when compared with gravimetric method as shown in the following table:

Summary of average difference between extraction and Babcock methods

	TYPE OF TEST BOTTLE		
	30 per cent 9 inch, 9 grams	50 per cent 9 inch, 18 grams	50 per cent 6 inch, 18 grams
Reading to top of meniscus	+0.62	+0.62	+1.00
Reading to middle of meniscus	+0.22	+0.19	+0.43
Reading to bottom of meniscus	−0.19	−0.21	−0.12
Value of total meniscus or per cent	+0.81	+0.83	+1.12

\+ Per cent higher than that obtained by the extraction method.
− Per cent lower than that obtained by the extraction method.

The table represents the average of seven lots of cream. It will be seen that neither readings, to top, middle or bottom of meniscus, gives the true per cent of butter fat. If we examine the table from which the foregoing averages were obtained, we find that the greatest variation from the gravimetric extraction method by reading to the top of the meniscus, was for:

9 grams, 30 per cent, 9 inch	0.79 per cent
18 grams, 50 per cent, 9 inch	0.88 per cent
18 grams, 50 per cent, 6 inch	1.53 per cent

The readings of the test reported by Webster were made by a trained chemist. No doubt a still greater variation in reading would have occurred had the reading been made by the ordinary cream tester. The method, proposed by Webster in 1904, was never generally used.

In 1908–1909, Eckles, at that time Chief of the Dairy Husbandry Department of the Missouri Agricultural Experiment Station, proposed the use of amyl alcohol to eliminate the meniscus in reading the Babcock test for fat in cream. This was at least suggestive though it was never generally adopted for the reasons that amyl alcohol dissolves butter fat and being miscible with water, gave low readings, and the vapor of amyl alcohol is unpleasant and in excess may act as a poison.

About the same time, the Wisconsin Agricultural Experiment Station suggested the use of fat saturated ethyl alcohol to eliminate the meniscus. This modification was an improvement over amyl alcohol and was quite generally used. This modification also had its objectionable features, mainly the alcohol was saturated with butter fat at room temperature and when coming in contact with the warm fat column (135-140°F.) in the Babcock bottle, dissolved some of the fat and not infrequently a cloudy zone was produced at the juncture of alcohol and butter fat, which interfered with accurate readings. The foregoing substances were highly suggestive of a possibility of finding a substance which would eliminate the meniscus and give accurate readings and free from the objectionable features of amyl alcohol and fat saturated ethyl alcohol.

In 1909–1910, Professor O. F. Hunziker, at that time chief of the Dairy Husbandry Department, Purdue University, outlined and conducted with his assistants, an unexhaustive investigation on the subject of cream testing by the Babcock method, Bulletin 145, Purdue University Agricultural Experiment Station.

The following table taken from Bulletin 145, gives the reading of the meniscus of 355 samples of cream.

TABLE 1

*Showing reading to bottom and to top of the meniscus, per cent, meniscus and gravimetric fat estimation**

SAMPLE NUMBER	NUMBER OF TESTS	READING THE FAT COLUMN		MENISCUS	GRAVIMETRIC FAT ESTIMATION
		To top of the meniscus	To bottom of the meniscus		
		per cent	*per cent*	*per cent*	*per cent*
1	16	16.62	15.62	1.00	15.90
2	36	26.61	25.58	1.03	26.37
3	40	21.42	20.42	1.00	20.95
4	39	13.38	12.38	1.00	12.86
5	40	18.43	17.59	0.84	17.65
6	18	21.00	20.50	0.50	20.10
7	40	13.29	12.29	1.00	12.41
8	24	21.96	20.96	1.00	21.14
9	11	25.93	24.90	1.03	26.18
10	11	25.29	24.29	1.00	24.52
11	35	30.50	29.50	1.00	29.85
12	21	29.96	28.96	1.00	29.40
13	24	39.65	38.23	1.42	38.40
Average....................		23.38	22.40	0.99	22.75
Total number of tests.......	355				

* Bulletin 145, Purdue University Agricultural Experiment Station, 1910, p. 582.

The results of this investigation as shown in table 1 on the value of the meniscus which to a great extent corroborate the results obtained by Webster. In the foregoing tests the 6 inch 9 gram 50 per cent cream test bottle was used. The lowest value of the meniscus for the average of 18 samples was 0.50 per cent. The highest value of the meniscus for the average of 24

samples of cream tested, was 1.42 per cent. The average value for 355 tests was 0.99 per cent.

From table 1 it will be seen that there is a great variation in the value of the meniscus when read under uniform conditions.

TABLE 2

*Showing a comparison of glymol readings with the results of the gravimetric fat estimations**

SAMPLE NUMBER	NUMBER OF TESTS	GLYMOL READING	GRAVIMETRIC FAT ESTIMATION	DIFFERENCE
1	11	40.62	40.31	+0.31
2	12	34.30	34.06	+0.24
3	12	31.65	31.71	−0.06
4	12	29.87	29.61	+0.26
5	11	45.02	44.80	+0.22
6	12	28.83	29.04	−0.21
7	12	27.65	27.48	+0.17
8	12	19.24	19.47	−0.23
9	12	20.04	20.00	+0.04
10	12	19.00	18.88	+0.12
11	6	32.62	32.29	+0.33
12	12	35.78	35.96	−0.18
13	12	17.46	17.61	−0.15
14	12	43.83	43.77	+0.06
15	12	39.76	39.62	+0.14
16	12	40.73	40.73	0.00
17	12	27.76	27.89	−0.13
18	12	21.56	21.72	−0.16
19	11	38.37	38.22	+0.15
20	9	19.87	20.06	−0.19
21	10	16.53	16.80	−0.27
22	12	20.85	20.83	+0.02
Average..................		29.66	29.584	0.02
Total number of tests.....	248			

* The above data were taken from Bulletin 145, Purdue Agricultural Experiment Station.

This showed that the principle defects of the Babcock method for cream testing was in the uncertainty of the reading of the fat column. These results suggested the desirability of using some liquid which would eliminate the meniscus and give concordant and accurate tests. During the preliminary experiments

various mixtures of hydrocarbon oils were tried. It was at this time when white mineral oil or glymol[1] was first used to eliminate the meniscus in the Babcock test. After being convinced of its superiority of any liquid which had been tried, 248 samples of cream were tested by the use of glymol. Also a gravimetric determination was made for fat of each sample. The method used for fat extraction was the paper coil method which was the official method in 1910. Detailed results of the experiment are shown in table 2.

Inasmuch as the cream tests in tables 1 and 2 were made by the authors of Bulletin 145, who used more than ordinary precautions, we deemed it desirable for this article to secure additional data on cream testing. This data to be secured under the usual creamery routine practice. The tester was not aware of the purpose for which the tests were to be used. The fat in the samples of cream used for these tests was determined by the Roese-Gottlieb method. These results are shown in table 3.

From the work of this station, as well as the work of other investigators, there is no question but that the meniscus is the principle cause of the inaccuracy. There are reasons why reading the meniscus, either to the top or bottom and making proper allowances, cannot give accurate results. First, the meniscus is very seldom well defined; second the effect of light, whether reflected or transmitted; third, the angle at which the test bottle is held affects the reading of the meniscus. Only under exceptional conditions can the meniscus be accurately read. The practice of adding one-third of the per cent of the meniscus to the reading when made to the bottom of the meniscus, has its serious faults, especially in the hands of the average cream tester. It was shown in Bulletin 145 that the per cent of meniscus varied from 0.50 to 1.56 per cent and this in the hands of men of experience and training.

In a very recent article published by Doan and coworkers (Dairy Science, Vol. VI, No. 5), on cream testing where some data are given. These authors in tests of 10 samples of cream

[1] A mineral oil corresponding to the liquid petrolatum U. S. P., is very satisfactory and is to be recommended.

show that readings to the bottom of the meniscus varied from −0.26 to +1.22 per cent as compared with the Roese-Gottlieb method and in three of the samples the error exceeded the limit of error allowed for the Babcock test bottles. These three readings being 0.59, 0.60 and 1.22 per cent higher than the gravimetric determinations. Doan and coworkers also make the following statement concerning the use of glymol (p. 407, l.c.) "Gregory and Hammond in the recent Purdue Circular No. 78 Revised, use Hunziker's method of including one-third the top meniscus or

TABLE 3

Showing the comparison of glymol reading when made under routine creamery testing

SAMPLE NUMBERS	GLYMOL READING	ROESE-GOTTLIEB METHOD	DIFFERENCE	SAMPLE NUMBERS	GLYMOL READING	ROESE-GOTTLIEB METHOD	DIFFERENCE
1	28.5	28.53	−0.03	13	32.0	32.17	−0.17
	28.5				32.0		
2	24.0	23.92	+0.08	14	30.5	30.42	+0.17
	24.0				30.0		
3	23.5	23.19	+0.31	15	30.5	30.53	−0.03
	23.5				30.5		
4	22.5	22.16	+0.09	16	36.0	35.67	+0.33
	22.0				36.0		
5	53.5	53.69	+0.06	17	31.5	31.90	−0.15
	54.0				32.0		
6	43.0	42.65	+0.35	18	27.5	27.49	+0.01
	43.0				27.5		
7	47.0	46.94	−0.19	19	26.5	26.21	+0.04
	46.5				26.0		
8	47.0	46.99	+0.01	20	36.0	35.95	+0.05
	47.0				36.0		
9	40.5	40.87	−0.12	21	36.0	36.10	+0.15
	41.0				36.5		
10	42.0	42.32	−0.32	22	29.0	29.43	−0.43
	42.0				29.0		
11	38.0	37.92	−0.17	23	27.5	27.19	+0.31
	37.5				27.5		
12	38.0	38.09	−0.09	24	25.0	24.87	+0.13
	38.0				25.0		
Averages.					33.969	33.967	+0.002

The differences represent the difference of the average of the glymol reading.

flattening the meniscus with glymol." This method of including one-third the upper meniscus was not recommended to equal the accuracy of the use of glymol in Bulletin 145. In the conclusion reached and expressed in this Bulletin, Summary 17, the following statement was made: "*For uniform and accurate reading of the test, the meniscus must be eliminated. This can be done by the use of glymol.*"

The subject of cream testing was exhaustively studied and findings presented in Purdue Bulletin 145, 1910. These results were accepted by the dairy industry as a decided advance in overcoming the difficulties in cream testing. The presentation of the data made under normal conditions and care, are the deciding factors regarding the value of any analytical method. Tables 2 and 3 both contain data obtained by the glymol method. By comparing these results it is evident that the glymol method has withstood the lapse of time and adverse criticisms as the new data confirm the old.

FIRST AMERICAN WORLD'S DAIRY CONGRESS A SUCCESS

J. H. FRANDSEN

The World's Dairy Congress which was held in Washington October 2 and 3, Philadelphia October 4, and Syracuse October 5 to 10, 1923, was attended by 231 delegates from 43 foreign countries, together with 1590 registered American delegates from 47 states and the District of Columbia, only one state not being represented; a total of 1821 registered delegates. Doubtless there were individuals who attended one or more of the 27 section meetings without registering.

Canada led with 100 delegates, England 22, Switzerland 12, Scotland 10, while the remaining 39 countries had from 1 to 9 each. Japan, Argentina, South Africa, and Russia are suggestive of the distant countries represented.

Of the United States delegation, New York State led with 313, Pennsylvania followed with 175, Illinois 133, District of Columbia 106, and in decreasing numbers down through the entire list of states.

The program listed 256 papers to be delivered at the 27 section meetings. Of these, 240 papers, including every phase of dairy science, practice and utilization were presented, 115 of these papers were contributed by foreign authors and were, with few exceptions, delivered in person by the author. Indications are that the printed proceedings of the Congress will consist of two volumes of approximately 900 pages. The editorial work is practically completed. The volumes will be published by the United States Department of Agriculture, and persons not registered as delegates or members of the Congress will doubtless be able to secure the proceedings in the same manner as other government publications, to the extent that they are available. Requests should be addressed to the

Superintendent of Documents, Government Printing Office, Washington, D. C.

The Congress was made possible by the contribution of from a few dollars to as much, in some cases, as one thousand dollars, by 402 organizations and individuals interested in the scientific and educational work of the dairy industry.

Some of the noteworthy comments on the results of the Congress may be summarized as follows:

So many men from so many countries can not come together and make acquaintanceships without being a real contribution to a better international understanding.

Many of our own people did not realize the high character of research work in progress in America until these reports and papers were heard at the same program with those from European scientists whose names have long been familiar to this country.

Foreign comment emphasizes the leadership of America in the sanitary supervision of its milk supply for large cities; its efficient pasteurization and wide distribution; the development of our ice cream trade.

One foreign paper, referring to our milk distributing and ice cream plants, says: "These are on a huge scale, almost quite unknown in these countries. So far, at any rate, as the American cities and large towns are concerned, distribution of milk in open vessels does not now exist." . . . . "It was not possible for any delegate to take in all that occurred at this Congress, but we saw sufficient to show us that the importance of dairying is receiving a large share of recognition from the governments and people of that country." . . . . "It would be impossible for us to say too much of the cordial manner in which we were welcomed everywhere we went and of the desire of all whom we met to impart such information as we desired."

Another foreign delegate, after returning home, states through the press: "The dairy industry in the United States is far more advanced than that of this country. They employ machines on a large scale, spend tremendous amounts of money in advertising, and have founded many colleges where every phase of dairy produce is dealt with. The

average American drinks twice as much milk as a Britisher; in fact, Americans are doing their best to produce an A-1 nation."

One delegate, commenting, says that where America is ahead of his country is in the successful way in which "the trade combines with educational and other authorities in propaganda to educate the children and the public as to the value and use of milk in the national diet.

One of the gratifying results was the recognition given the Congress by the President of the United States, the cabinet officers and the American metropolitan press. This is a real contribution to industry consciousness, a stimulus to closer understanding of the immense number of individuals engaged in a varied industry of science, education, business, production, manufacture, distribution and use.

Mention of the World's Dairy Congress would not be complete without a word of tribute to the two men who more than any one else made this undertaking the splendid success that it was. To President H. E. Van Norman and to Program Chairman L. A. Rogers the American Dairy Science Association owes a debt of gratitude. The first American Dairy Congress was a distinct success.

THE EFFECT OF STORAGE ON THE PEROXIDASE ACTIVITY OF WHOLE MILK POWDERS[1]

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In a previous paper (1) the writers reported that certain milk powders exhibited greater peroxidase activity than others. This observation was made on fresh powders. The enzyme was inactive in case of drum-made powders dried under atmospheric pressure, but active when dried under reduced pressure or in a vacuum. Spray-made powders showed pronounced evidence of the presence of the enzyme. The difference in peroxidase activity in fresh powders is due largely to the degree of heat to which the liquid milk has been subjected in process of manufacture. It has also been observed by the writers that old powders in most instances showed but slight enzyme action; that the peroxidase activity decreased with the age of the powder.

In the present paper the investigation was carried on in connection with an experiment on the keeping quality of whole milk powders. The samples were stored under various conditions which made possible the studying of several factors influencing the peroxidase activity.

POWDERS STUDIED

The powders studied included Creamon, a certrifugal spray-made powder made by the Dick process; Klim, a pressure spray powder made by the Merrill-Soule Company; and Creamora A, an atmospheric drum-made powder made by the Dry Milk Company of New York.

[1] Published with approval of the Director as paper no. 411, Journal Series, Minn. Agric. Exp. Station.

TEST USED

The Storch test was used to denote the activity of the enzyme studied. It should be remembered that this test is not a quantitative test. A fresh supply of reagents was made up each time the samples were tested, as an interval of three months elapsed between each test. The powders were reconstituted with water to resemble normal milk in total solid content and the Storch test made on a 10 cc. sample.

FACTORS STUDIED

The factors studied which were under suspicion as influencing peroxidase activity included moisture and air, air, storage temperature, age of powder, and containers in which the powders were stored.

The effect of air and moisture

Before studying any factors a test was made of the fresh powders and it was found that the Creamora A powder showed no peroxidase activity, so it was not included in the present study.

Samples of Creamon and Klim were exposed for three hours at room temperature to an atmosphere partially saturated with moisture. The humidity of the atmosphere was 70 per cent. The moisture content of the powders was increased from 2.98 per cent to 4.31 per cent in case of the Creamon powder and from 2.57 per cent to 4.67 per cent in the case of the Klim powder. These samples gave positive Storch tests after the treatment, but the activity was slightly less than prior to this treatment. The powders were packed into two ounce screw top ointment jars (opaque glass) and placed in storage at 4°C., 20°C., and 37°C. and observed after three, six, nine, and twelve months (see tables).

The treatment received by the powders prior to storing was very detrimental to the peroxidase activity. After three months in storage there was not a trace of peroxidase. The same treatment was very conducive to tallowiness of the fat and insolubility of the powder. Powders stored at temperatures as high as 37°C. were highly discolored by the heat.

That air alone was a factor may be seen in the case of samples 1, 2, and 4 (tables). In number 1 the powders were milled to

TABLE 1

The peroxidase activity in a sample of Klim powder stored under various conditions

	STORAGE TEMPERATURE °C.	PEROXIDASE REACTION			
		After 3 months	After 6 months	After 9 months	After 12 months
(1)					
Powder milled in ball mill 1 hour and 25 minutes to break down air cell within the powder grains	20	+	+	±*	−
Packed in 2 ounce screw top ointment jars (opaque glass)	37	−	−	−	−
(2)					
Powder untreated and packed in 2 ounce screw top ointment jars (opaque glass)	20	+	±	−	−
	37	−	−	−	−
(3)					
Powder exposed 3 hours to partly saturated atmosphere, packed in 2 ounce ointment jars (opaque glass)	4	−	−	−	−
	20	−	−	−	−
	37	−	−	−	−
(4)					
Powder untreated, packed in 2 ounce ointment jars, without covers. Placed in dessicator and 29 inch vacuum drawn	20	+	+	+	±
(5)					
Powder untreated, packed in (Sealright) paper container	4	+	−	−	−
	20	+	±	−	−
(6)					
Powder untreated, placed in (Doubletite) containers	20	+	+	±	±
	4	+	+	±	±
	37	+	−	−	−

* ± Indicate that the activity was faintly noticeable.

expel the air within the particles. The milling destroyed the air cell. Number 2 was packed and stored without treatment, while a vacuum of 29 inches was drawn on powder in number 4. It

TABLE 2

The Peroxidase activity of a sample of Creamon powder stored under various conditions

	STORAGE TEMPERATURE °C.	PEROXIDASE REACTION			
		After 3 months	After 6 months	After 9 months	After 12 months
(1)					
Powder ground in ball mill 1 hour 20 minutes to break down air cell within the grains	20	+	+	±	−
Packed in 2 ounce screw top ointment jars (opaque glass)	37	−	−	−	−
(2)					
Powder untreated and packed in 2 ounce screw top ointment jars (opaque glass)	20	+	+	−	−
	37	−	−	−	−
(3)					
Powder exposed 3 hours to partly saturated atmosphere. Packed in 2 ounce screw top ointment jars (opaque glass)	20	−	−	−	−
	4	−	−	−	−
	37	−	−	−	−
(4)					
Powder untreated, packed in ointment jars without covers. Placed in dessicator and 29 inch vacuum drawn	20	+	+	+	±
(5)					
Powder untreated. Packed in (Sealright) paper containers	4	+	−	−	−
	20	+	±	−	−
(6)					
Powder untreated. Placed in tin (Doubletite) containers	20	+	+	±	±
	4	+	+	+	+
	37	−	−	−	−

will be observed that the peroxidase was much more active in sample 4 than in 2. The peroxidase of sample 1 was slightly more active than that of sample 2.

The influence of temperature

The temperatures at which the powders were stored was an important factor influencing the activity of peroxidase.

All samples except one stored at 37°C. gave a negative Storch test when examined after three months. Those stored at 4°C. and 20°C. were active after three months except the powders exposed to moist air before placing in storage. The heat at 37°C. greatly accelerated oxidation when air was present and caused an earlier destruction of the peroxidase. The Klim sample which was stored in the "Doubletite" tin container showed positive peroxidase after being stored for three months at 37°C. The reason for the activity of the enzyme in the Klim sample in this container was undoubtedly due to the small amount of air contained within the package. Creamon contains more air in a given package because of the large air cell within each individual grain, and the inability of the powder to pack closely because of the relatively large size of the grains.

Influence of time or age of powders

That time was a factor in the activity of peroxidase enzyme was clearly shown. There was a gradual decrease in the activity as the time advanced. This change was influenced by such factors as air, moisture, and temperature. When the powders were stored under vacuum there was still activity after one year. This was slight, however. Peroxidase activity was very slight after six months in storage under the best conditions.

The influence of containers

The containers in which the powders were stored played an important rôle. It was found that the containers which prevented the entrance of air and moisture gave the best results. A particular tin container known as "Doubletite" gave the best results. The activity of the enzyme studied was in evidence, though slightly, after one year in storage at 4°C. and 20°C.

Paper containers were not efficient, neither were the screw top ointment jars. It was impossible to prevent the entrance of air and even moisture when these containers were used.

SUMMARY

Factors known to favor oxidation, such as air, heat, and moisture, proved detrimental to peroxidase activity in the powders studied.

Powders stored under vacuum, and powders stored in containers which prevented the entrance of air and moisture, showed greater peroxidase activity than samples of the same powders stored in containers which permitted the entrance of air and moisture.

Increasing the moisture content of the powders by exposing them to atmosphere nearly saturated with moisture was very detrimental to peroxidase activity. No activity could be detected after three months in storage at 4°C., 20°C., and 37°C.

High storage temperature and high moisture content greatly accelerated the rate of destruction of the enzyme studied.

ACKNOWLEDGMENT

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THE COLOR OF COW'S MILK AND ITS VALUE

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Milk is an almost opaque fluid, made up of fat globules in temporary emulsion, colloidal casein and albumin, and various salts, sugar and albumin in solution. It varies in color, due to various reasons, from a yellowish white to nearly white or even to a bluish tinted white.

The observed color is a composite of many contributing factors. The chief of these is the yellow fat globules which are responsible primarily for the yellowish tints. When fat rises as cream most of the yellow color shows above the cream line. The size of these fat globules in the milk influences the composite color as well as does the color of the globules themselves (1), for the surface of small globules is greater in proportion to their size than the surface of the larger ones. Since light is reflected in all directions by these surfaces, milk with smaller fat globules would have a greater reflection and a greater dispersion of reflection, causing a whiter milk, even though the total amount and color of the fat and all other conditions were equal.

The colloidal ingredients also influence the opacity and whiteness of milk depending on the size of particles and the amount of these ingredients present.

As has been stated the greatest influence on color is produced by the fat. Where the fat of different milks is the same color, the yellowishness of the milk will roughly run in proportion to the amount of fat present. But the yellowness of milk fat itself is very different as between different milks so that in comparatively few cases should the above proportion be true.

Palmer and Eckles (2) have shown that the pigment in milk fat is carotin associated with a small amount of xanthophylls and that these pigments are the same as those found in green and yellow vegetation. Furthermore that the pigments are ingested with the food and secreted apparently unchanged in the milk fat. They were able to increase or decrease the color of the fat, at will, by changing the amount of pigmented food in the ration. This readily explains why summer milk fat is high in color and winter milk fat low in color. In the spring and early summer the cows usually have access to pasture or are fed considerable quantities of green feeds which contain comparatively large amounts of carotin; while in the fall and winter the cows are usually stall fed on hay, silage and grain containing comparatively small amounts of the pigment, depending to some extent on how bleached the hay is.

Two cows fed at the Maryland Station gave fat with 5 units of color on a ration of silage, beet pulp, and grain. When 20 pounds of green rye were added to the ration the color of the fat increased to 30 units, or approximately 6 times the depth of color, in the course of two weeks. This necessarily had a very pronounced effect on the color of the milk notwithstanding the fact that, the percentage of fat was greater at 5 units than at 30 units.

It is a well known fact that colostrum is exceedingly high colored. This is principally due to the intense color of the fat at this time. While colostrum is little different in fat content from normal milk, the greater amount of lipochrome in the fat has the power of deepening the color. In the cows noted previously, the color of colostrum fat was equivalent to 43 units on the day following parturition while six days later the color of the fat had fallen to 5 units in the case of one cow and to 5 units after sixteen days in the case of the other. This high color is not entirely dependent on feed, for in this case the feed had been rather constant over quite a period, consisting of timothy hay, corn silage, beet pulp and grain (a low pigmented ration) for sixty-five and forty-nine days, respectively. It is not definitely known why the colostrum fat is so highly colored, but it is probably due to a storing of the pigment in body fat laid on during the period

of non-milking; with a subsequent katabolism of these fats and their secretion in the first milk after calving. It may also be partially due to the absorption of the corpus luteum (yellow body), the pigment of which Palmer and Eckles (2) have shown to be largely carotin.

From these observations we can say that primarily the yellowishness of milk depends on: first, the color of the fat, or the amount of so called lipochrome present in the fat; and, second, the percentage of fat present, recognizing of course that other factors do influence the observed color to a minor degree. A factor of minor importance which has not been mentioned is the color of the milk serum or whey. It is an observable fact that milk coagulated for cheese making leaves a greenish-yellow whey. This color is due to a pigment known as lactochrome which Palmer and Eckles (2) found to be identical with the normal urine pigment, urochrome. It is not known what produces the variations observed in the color of the serum but variations which influnece slightly the color of the milk do occur.

Color in milk is a point of some commercial value, the average consumer preferring a yellowish milk, thinking it to be richer in cream (butter fat) than whiter milks. While this is true in general of milks where the pigmentation of the fat is approximately equal, it does not hold in the majority of cases. The demand for yellowish milks should however be encouraged for quite another reason.

It was at one time suggested that carotin and fat-soluble—A vitamin were identical or at least always found associated. While this theory has been disproved by Palmer and Kennedy (3), it is still a fact that milk from cows fed on ample quantities of green vegetation is higher in vitamin content than milk from cows fed on bleached hays, silage, most roots and grains (4). Thus it follows that summer milk is richer in vitamins than winter milk under prevailing conditions of production. Furthermore a highly pigmented milk fat indicates quite accurately the feeding of materials ordinarily considered rich in vitamins. From these facts it would seem that milk containing a high colored fat would

carry a comparatively large amount of vitamins. But on the other hand, in consideration of Palmer and Kennedy's (3) results, a low colored fat would not necessarily indicate that such a milk was low in vitamin content, although in a large majority of cases such would undoubtedly be true under commercial conditions. In this discussion the references to vitamins apply more particularly to vitamin A, although it is believed that the statements would still hold true for vitamins B and C as well.

COLOR INSTRUMENTS

An instrument has been devised by Prof. R. W. Wood of the Johns Hopkins University, for measuring the color of milk. This instrument compares the color of light transmitted through a layer of milk 1 cm. in thickness with the color of the same light passing through a wedge of yellow glass and falling on a white surface. A match is secured by changing the position of the yellow wedge, the thick end of the wedge giving a more yellow color on the white surface than the thin end. The instrument is arranged so that not only the depth of color of the light falling on the white surface may be altered, but also the amount of light allowed to fall on this surface. The latter is regulated by adjusting the slit, through which the light passes, by means of a thumb screw. The readings are made by noting at what point on the wedge a color match is secured, the wedge carrying a scale graduated from 0 to 15 in centimeters, being subdivided into millimeters.

This method of measuring color seems not to be entirely accurate since it has been noted, in some cases, that whole milk, and the skim milk from this whole milk, give the same reading; while comparison in test tubes show the whole milk considerably more yellow than the skim milk.

An instrument for measuring the color of milk fat and of milk serum is the Lovibond tintometer which was used by Palmer and Eckels (2) in their work. This instrument is a colorimeter making use of colored glasses as the standard. The glasses are furnished in graduated units of three colors—red, yellow and blue. Various combinations of these three primary colors will produce

almost any tint of any color. Since in milk fat the ratio between yellowish tints and reddish tints is not constant it is difficult to find a solution which can easily be used as a standard. Potassium dichromate can perhaps best be used, but the use of standard glasses is much simpler and far easier to manipulate.

In preparing the fat for a color reading, at least one quart of milk is churned in a small glass churn, the resulting butter rendered at 60°C. to 70°C. and the curd removed as completely as possible by centrifugation in a separatory funnel, washing with hot water. The fat is then filtered, while hot, through paper on a warm water funnel, or in an oven at 60°C. to 70°C. and the filtrate put into a cell ½ inch deep. This one-half inch of fat is then matched in the tintometer with the standard glasses which are numerically graduated according to depth of coloring, usually about 1 unit of red being required to 10 or 15 units of yellow for a perfect match. The number of units of both yellow and red used is the color reading of the sample. For instance: yellow, 25 units; red, 2.1 units. This method of ascertaining the color of the milk fat and indirectly the nature of the ration being fed the cow, is superior to the direct readings on the milk because it does away with other factors not directly concerned. Either method is an arbitrary one but the readings on the milk itself are rendered higher or lower, due to the amount of fat present, while those on the fat proper are not in error in this regard. To show the changes in fat pigmentation due to alteration of rations, two cows (previously mentioned) were fed the same rations, and color readings made on their milk fat over a period of several months with results shown in the table. The rations consisted of concentrates, and beet pulp, which were practically constant during the entire period. Hays, corn silage, soilage crops and green corn were added or removed from time to time as noted opposite the date.

Readings on two cows fed the same ration

cow 324			DATE	CHANGES IN FEED	cow 267		
Test	Fat color				Fat color		Test
	yellow	red			yellow	red	
4.50	4.5	0.5	April 16		5.0	0.8	5.40
4.40	10.0	1.0	April 23	Green rye	7.3	0.8	5.45
4.50	17.2	1.1	April 30		10.0	1.0	4.35
4.50	29.0	1.0	May 7		10.0	1.0	3.80
4.50	24.0	1.1	May 14		11.4	1.1	3.75
4.45	16.0	1.3	May 21		15.0	1.2	3.80
4.40	20.0	1.5	May 28	Green veitch	30.0	1.5	3.65
4.50	Lost		June 4		Lost		3.60
4.65	30.0	1.3	June 13		20.0	1.2	3.80
4.55	23.0	1.0	June 18		25.0	1.0	3.65
4.40	32.0	1.0	June 25		30.0	1.0	4.00
4.30	31.2	0.7	July 2	Green hay	22.2	1.1	3.55
4.65	19.9	0.9	July 9		25.0	1.0	3.65
4.60	29.0	1.2	July 16		25.0	1.2	3.60
4.50	31.5	1.0	July 23	Green corn	30.0	1.1	3.95
4.60	29.0	1.1	July 30		24.0	1.3	3.85
4.40	26.0	1.3	August 6		15.0	1.2	3.60
4.60	25.0	1.5	August 13	Green alfalfa	20.0	1.4	3.90
4.35	26.8	1.5	August 20		20.5	1.5	3.70
4.30	28.5	1.7	August 27	Yellowing hay	18.1	2.0	3.60
4.40	21.1	1.6	September 3		17.2	1.5	3.60
4.40	17.7	1.7	September 10		16.2	1.4	3.90
4.35	29.0	1.5	September 17	Sorghum corn	22.2	1.2	3.65
4.80	29.0	1.3	September 24	Green soy beans	19.0	1.4	4.30
4.25	20.0	1.5	October 1		23.0	1.2	4.00
4.90	30.0	1.5	October 8	Cured timothy	21.0	1.1	4.20
4.80	17.2	1.3	October 15	Silage	11.0	1.0	4.20
5.30	11.0	1.3	October 22		6.0	1.0	4.60
5.50	11.0	1.1	October 29		5.0	0.9	4.40
5.60	6.5	0.7	November 5		4.7	0.8	4.60
5.75	4.1	1.0	November 12		5.0	0.8	4.60

In a study of this table two things stand out, namely, that all the green roughages produced milk fat with a comparatively large amount of pigment and the lack of green roughages gave rise to milk fat which was comparatively low in pigment. In several cases the change from one green roughage to another seemed to lessen the color of the milk fat temporarily. The decline in color after October 8 is striking, when cured timothy

and silage were substituted for green soy beans; as is also, the rise in color when green rye was substituted for silage just previous to April 30. Throughout the period, cow 267 gave almost twice the amount of milk given by cow 324. This explains the lower color readings on her milk fat. The discrepancy in color of milk fat for cow 324 May 21 and May 28 can be partially laid to the fact that she was "off feed" during some of this time.

While this work is largely a preliminary observation, the results do show that the pigmentation of the fat, roughly runs parallel to the amount of green feeds assimilated.

In a final analysis it may be found that the color of cows milk or the color of milk fat is of no great importance, but from our present knowledge color does seem to be an indication of the vitamin A value of milk used for a food and possibly an indication of the presence or absence of other vitamins as well. This statement, applies only to milk produced under present commercial conditions.

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TRUE PROTEIN VERSUS CRUDE PROTEIN AS A BASIS FOR COMPUTATION OF FEEDING STANDARDS

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First let us understand that a feeding standard is not a statement of absolute scientific fact, but a device of convenience, based upon rather generalized scientific truths, and intended as a guide in practical animal feeding.

Feeding standards, of course, may set forth nutritive requirements of any kind whatsoever, but as acutally used they all represent, in terms of some sort, the two factors of nitrogenous and non-nitrogenous organic nutriment.

The method of statement of the first-mentioned factor, of nitrogenous nutriment, is the subject of this consideration.

As we conceive this problem it is, in reality, not one of evidence for the establishment of scientific truth, but rather one of analysis of a situation depending upon recognized facts, for the determination of the most useful of two conventional procedures.

The justification for this discussion lies in the fact that within comparatively recent years our understanding of the whole subject of protein metabolism has undergone revolutionary change, and this subsequent to the establishment of the current usage of the two methods of expression of the protein component of rations.

This subject was discussed by Armsby as late as 1917,[1] and from his presentation we quote the following:

For the present the only available measure of the protein values of feeding stuffs is the total amount of digestible protein which they contain. In the application of this method it becomes necessary to decide

[1] Armsby: The Nutrition of Farm Animals, 1917, pp. 684–688.

whether the basis of comparison shall be the "crude" protein or the "true" protein as determined by existing conventional methods; in other words, to decide what value, if any, shall be assigned to the non-protein.

It would appear that the value of the non-protein of a feeding stuff as a source of body protein must be determined by precisely the same thing which is believed to measure the value of an individual protein or of the mixed proteins of feeding stuffs, viz., the kinds and proportions of amino acids which it can yield, since there is no evident reason why an amino acid existing already formed in a feeding stuff should differ in value from the same substance split off from protein in the process of digestion. If this be admitted, however, the distinction made in recent years between protein and non-protein in feeding stuffs becomes rather meaningless. If the value of each is measured by its amino acid content, then what is needed to fix the production values of feeding stuffs as regards protein is a knowledge of the kinds and amounts of these compounds which the feeding stuff as a whole (i.e., its crude protein) can furnish, irrespective of whether they exist in a soluble, as it were predigested, form or are first produced in the digestive tract of the animal.

The conclusions of Armsby, in full,[2] are as follows:

"It seems clear that the evidence is insufficient to warrant any general conclusions regarding the nutritive value of non-protein, if indeed any general statement regarding such a heterogeneous group is possible. Ultimately, it may be that studies of the amino acid yields of the total nitrogenous matter (crude protein) of feeding stuffs, or comparisons of its relative efficiency in supporting maintenance or growth, will lead to the formulation of production values for the crude proteins of different materials, but for the present the writer feels that the safer course is to make the digestible "true" protein, so-called, the basis of comparison.

"While some experiments, notably the Copenhagen experiments on dairy cows, seem to indicate a relatively high value for the non-protein of roots especially, most investigators, particularly Morgen and his associates, have, as already noted, found them decidedly inferior to protein. It is true that the non-protein contains amino acids which may at times be utilized indirectly by herbivora through the agency of

[2] Loc. cit., p. 687.

the microörganisms of the digestive tract, but even this indirect utilization seems to be rather limited in extent in most instances. The conventional "true" protein, on the other hand, may be regarded as representing approximately the real proteins of a feeding stuff and it would seem that these mixed proteins are likly to supply more nearly a balanced amino acid mixture in digestion than would result from the inclusion of the non-protein. Investigations of the protein values of feeding stuffs should doubtless take account of whatever amino acids the non-protein supplies, i.e., they should relate to the crude protein. With continued study of these relations, it may be hoped that greater clarity may be attained, but until that end is reached, the digestible "true" protein seems the safer basis for the formulation of tables of the production values of feeding stuffs and for the computation of rations.

Whatever error is thus involved tends to make the protein content of the rations somewhat higher than if the crude protein were made the basis of the computation. It is, therefore, an error on the safe side, since a deficiency of protein may limit production while a surplus at worst simply tends to increase the cost of the ration, and the difference in the latter respect is seldom considerable.

In discussing the same matter Henry and Morrison[3] say, in part:

Whether these amids can be used for the same purposes in the body as are the proteins of the food, has long been a disputed question. If the mixture of amids in a feeding stuff contains the proper proportion of the various amino acids (the protein building stones), it now seems certain that these amids can be used the same as the true proteins.

For example, about one-third of the crude protein in legume hays usually consists of amids. Nevertheless, the crude protein in these feeds is much more efficient for maintenance, growth, or milk production than the crude protein of the cereal grains, which contains but a very small proportion of amids. Similarly, about half the crude protein in corn silage consists of amids, largely formed by the breaking down of proteins in the ensiling processes, for amids form only about 15 per cent of the crude protein in dried corn fodder. Yet on the dry basis, corn silage is more valuable than corn fodder for stock feeding.

From these facts it appears logical in making up balanced rations for stock, to base the computations on the total amount of digestible

[3] Feeds and Feeding: Henry and Morrison, 1923, p. 64.

crude protein, as is advocated in the Morrison feeding standards. In view of our present knowledge it seems unwise to ignore entirely the value of the amids as sources of nitrogen for body uses, as is done in the Armsby and Kellner feeding standards.

A careful examination of this whole question reveals within it boundless possibilities for inconclusive discussion, for the reason that it is essentially like an incomplete mathematical problem, being unsolvable on a scientific basis.

The salient points in this situation are the following:

The distinction between protein and non-protein nitrogen has largely lost its significance, in the light of the newer knowledge of protein metabolism. In different feeds there is no uniform relation between non-protein and true protein nitrogen, either as to kind or as to quantity. Non-protein nitrogen is so heterogeneous in its nature—so lacking in uniformity of composition and nutritional value, that it is impossible to speak of non-protein, or to compare true protein and non-protein, in general terms, with accuracy or satisfaction. Some true proteins, because of a lack of essential amino acids, may be of limited value as protein; non-protein may be composed of varying combinations of useful and useless amino acids and amids, as well as a diversity of other nitrogen compounds—nitrogenous extractives, nitrogenous lipoids, nitrogenous glucosids, alkaloids and organic bases, as well as nitrates and ammonium salts. The nutritive value of non-protein nitrogen to contribute to the protein requirement is determined, then, just as is that of protein, by its individually characteristic amino acid composition, and by the demand for the several amino acids, as varying with and depending upon the feed combination in which it is used, and the particular nutritive requirements of the animal involved.

It should be noted that Armsby did not deny the nutritive value of non-protein nitrogen, in fact he definitely advocated[4] the consideration of the amino acid content of the non-protein in studies on the protein values of feeding stuffs; but at the same time he expressed the belief that until greater clarity is reached,

[4] Loc. cit., 687, lines 33 to 40.

as to the value of non-protein nitrogen "the digestible 'true' protein seems the safer basis for the formulation of tables of the productive values of feed stuffs and for the computation of rations."

Further, he made his position regarding this matter still more clear[5] by explaining that so long as the protein requirements of animals and the protein contents of feeds are both stated in the same terms, it will commonly make no significant difference whether this be on the relatively low level of true protein or on the higher level of crude protein figures.

It seems to me impossible to take serious exception to this point of view, and I believe that the underlying facts are understood alike, all the way around.

It is my belief, therefore, that there is in this problem no ground for important scientific difference, but merely slight dissimilarities of feeling as to evidence which is understood in practically the same way by every one.

But there remains the difference in usage—which, from a practical point of view is important, since results of experiments can be compared only if stated in the same terms, and since there is no convenient and accurate way of computing from one of these standards to the other.

The matter under discussion has to do especially with animal feeding. Protein figures in the literature of animal feeding are predominantly on the "crude" basis. Is there practical advantage, therefore, in changing to the "pure" protein basis; and is there warrant for a dual form of statement?

The element of conventionalism is so prominent in both the true protein and the crude protein conceptions that a choice between them seems essentially unimportant—but there remains the practical consideration of the use of the literature.

To the man who is inclined to look deeply into the subject the "true" protein conception must seem less conventional, more scientific, and more satisfactory than the idea of "crude" protein, for the reasons that "true" protein is essentially more of one sort,

[5] Loc. cit., 325.

with a more definite and a more uniform nutritive value, than is "crude" protein. In my opinion, however, the difference is not one of importance in connection with the usefulness of data stated in these terms.

The scientific student, in the field of protein metabolism, will think in terms of amino acids. The true protein of a feed does not contain all of its amino acid fraction, while the crude protein contains all—and much else—some of related character—some not related.

All things considered, the writer favors the continuance of the use of the crude protein standard in the literature of animal production, and a separate statement of the true protein wherever it will be effective as a step toward recognition of the one fact of outstanding significance in this relation—that the nutritive value of protein, as protein, depends essentially upon the amino acids which it contains.

In the more critical sense the proteins of feeds can be compared only in terms of the amino acid balance of the rations in which they are used. This attitude being impracticable, for the present, at least, especially in connection with feeding standards, we have no recourse other than to make use of the highly conventional standards which we have discussed. In this connection, however, a very great improvement in the significance of protein values seems quite possibly attainable through our learning to determine the true digestibility of protein; when, indeed, we shall have done so. The writer believes that this is a promising subject for research, and that it should be vigorously prosecuted.

STUDIES IN THE GROWTH AND NUTRITION OF DAIRY CALVES

X. SELF-FEEDING A GRAIN MIXTURE TO YOUNG CALVES

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The use of the self-feeder in the raising of dairy calves has already been discussed in papers VII and VIII of this series, so it will not be necessary to go into the history of the work here. In the two previous papers a trial of sixty days' duration with young calves was reported and also an attempt to raise heifers to producing age with the aid of the self-feeder. In each case the animals were given a choice of grains and were allowed alfalfa hay at free will while milk was hand fed.

EXPERIMENTAL WORK

In the trial reported here 4 young calves, averaging sixteen days in age, were used. Information concerning them is given in table 71 and where necessary it is calculated to the day on which the trial started, namely, October 6, 1921.

The trial lasted for six periods of thirty days each. The calves were started on whole milk, hand fed, according to their apparent needs, and in the third period the substitution of skim milk started, though it was not absolutely completed until the last period.

Good alfalfa hay was kept before the calves at all times and they were provided with a mixture of 5 parts cracked corn, 2 parts ground oats, 2 parts wheat bran and 1 part old process linseed oil meal, by weight, in a self-feeder. Salt was provided at free will and the animals were watered twice daily.

TABLE 71

Animals used

NO.	CALF 565	CALF 570	CALF 572	CALF 573
Breed	Jersey	Ayrshire	Jersey	Ayrshire
Birth weight, pounds	51	55	41	58
Age, days	32	18	8	8

TABLE 72

Total feed comsumption for the group

PERIOD	WHOLE MILK	SKIM MILK	ALFALFA HAY	GRAIN MIXTURE
	pounds	*pounds*	*pounds*	*pounds*
I	1056		27	39
II	1210		107	147
III	1206	140	160	319
IV	728	820	221	483
V	48	1770	162	623
VI		1880	180	806
Total	4248	4610	857	2417
Average per calf	1062	1153	214	604
Daily average per calf	5.9	6.4	1.2	3.4

TABLE 73

Average live weights and body measurements

TIME	LIVE WEIGHT	HEIGHT	DEPTH	WIDTH
	pounds	*inches*	*inches*	*inches*
Start of Trial	59	25.6	10.9	6.0
End of period I	97	27.9	12.0	6.9
II	141	30.5	13.6	8.1
III	183	33.1	15.3	9.1
IV	239	34.6	16.9	10.0
V	297	36.2	17.7	11.0
VI	346	38.3	18.7	12.2

It will be noted that the grain consumption is heavy, being at all times greater than the hay consumption. On the average each calf consumed 3.4 pounds of grain daily and only 1.2 pounds of alfalfa hay.

The live weight of each animal was obtained at the end of each thirty-day period. The body measurements, height at withers, depth of chest and width at hooks were also determined. The animals showed excellent growth. This is more readily realized when the rates of gain are compared with those reported for normally fed heifers in paper III of this series. The normally fed heifers have an advantage in age of fourteen days in the comparison with the self-fed calves and yet at the end of the sixth thirty-day period, when the trial ended, the self-fed heifers showed increases of 491, 37, 56 and 77 per cent in live weight, height, depth and width, respectively. The corresponding increases or the heifers fed normally were 309, 34, 57, and 67 per cent.

The self-fed heifers showed good gains, but the cost of the gains is a very important factor. Using the same feed costs as were used in the other papers of this series it is found that the cost of feed for the normally fed heifers up to six months of age was $35.57, and for the self-fed heifers that had a choice of grains it was $35.81, while for the heifers in the work reported here, where a grain mixture was used the cost was $29.96 or a considerable reduction.

If the cost per pound of live weight gain be considered it is found that it amounts to 14.4 14.1 and 10.4 cents per pound for the various groups in the order mentioned above. This would indicate that calves can be raised economically when self-fed a mixture of grains. This is due largely to the fact that the amount of high priced feeds such as oil meal are limited and cheaper grains, such as oats, form a larger part of the ration.

SUMMARY

From the work reported here it would appear that dairy calves can be fed economically until six months of age with a self-fed mixture of cracked corn, ground oats, wheat bran and linseed oil meal. The proportions of the feeds in the mixture can be adapted to ruling prices. Alfalfa hay can be self-fed and the milk hand fed according to the requirements of the individual calves.

ASSOCIATIVE ACTION AS A CAUSE OF YELLOW COLOR IN YEAST COLONIES

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INTRODUCTION

During the past few years many samples of milk, cream and butter have been examined for the general types of yeasts contained by plating on whey agar, 1 cc. of a 1 per cent solution of tartaric acid being added to each plate at the time of pouring to restrain bacterial growth. While most of these samples came from the neighborhood of Ames, a considerable number were from other points in the state and some few from outside the state. Among the colonies observed the production of colors other than pink was extremely rare. The results herein reported deal with the presence of a yellow color, as a result of associative action, in colonies that were apparently yeasts but in which the effect of mold growth was evidently important.

EXPERIMENTAL

A canary yellow colony that was apparently a yeast was observed on a whey agar plate showing a growth of both yeasts and molds. Examination of the colony under the low power showed the structure to be that common to yeasts and a stained preparation revealed only budding cells so a tiny bit of the colony was shaken up in a water blank and whey agar plates poured for purification. After incubation at room temperature the plates showed numerous yeast and bacterial colonies but none of them were yellow and none became yellow even after prolonged holding.

At a later date a whey agar slope inoculated from a plate poured for a yeast and mold count on storage butter showed a bright yellow growth, and a microscopic examination of this proved the presence of both yeast and bacterial cells. The slope also showed a mold growth covering a good portion of the surface; the mold was probably carried over from the plate since this was heavily seeded and showed various types of yeast and mold colonies. A small amount of the yellow material from the slope was shaken up in a water blank and plated out and yeast and bacterial colonies quickly developed but all were white with no suggestion of a yellow color. Although the presence of bacteria in cultures of yeasts picked from plates is very common as a result of the bacterial cells being carried along by the yeast cells, it seemed advisable to make mixtures of the yeast and the bacterium with the idea that the color production might be the result of associate action between these two organisms. A considerable number of such mixtures were made on whey agar slopes but all of them failed to develop a yellow color.

Later observations of the plates from which the bacterial and yeast colonies had been picked showed the relationship necessary for the development of color. Two mold colonies that were probably carried over from the original slope had started to develop at some distance apart and where the molds had come in contact with or close to the yeast colonies, which by this time were up to several millimeters in diameter, the yeast colonies had assumed a bright yellow color. It was common to find only the part of a yeast colony toward the mold showing the yellow color, although eventually the entire colony became yellow. In some instances spore stalks later came out of the yeast colonies so it seems that the mold must have grown through them. A pure culture of the mold (mold 1) and a number of the yeast (yeast 1) were secured and used in various inoculation experiments.

By inoculating yeast 1 on a whey agar slope and, after a good growth had developed, inoculating mold 1 near it, a yellow color was regularly secured in the yeast growth when the mold had come near to or in contact with it. The continued development of the mold soon resulted in the yeast growth being entirely covered up so that the typical yellow color was no longer evident.

A yellow color in the yeast growth could be obtained by inoculating the yeast and mold at the same time or even by inoculating the mold first but when the yeast growth was heavy, as occurred when the yeast was inoculated first, the appearance was the most striking.

Although a yellow color was readily produced on whey agar slopes, plate cultures were more satisfactory as a means of showing the color production by the action of the two organisms. Plates showing a considerable number of colonies of yeast 1 were repeatedly inoculated at one or more points with mold 1 and the development of the yellow color followed. As the mold spread, a yellow color appeared in the nearby colonies—usually on the side toward the mold first—and then as the mold growth increased more distant colonies became yellow. A considerable number of colonies were cultured soon after they had become yellow by dipping a sterile needle into them and inoculating on whey agar slopes. In the inoculations molds and yeasts were both evident in most cases while in others only yeasts were found and these of course gave a white growth; from these results it seems that the mold growth in the yeast colonies does not necessarily need to be heavy for the yeast colonies to assume a yellow color.

As already suggested, the portion of the yeast colony toward the mold was most likely to show a yellow color first. In some instances the colonies were yellow mainly along a considerable portion of the edge so that the yellow area was shaped like a horse shoe.

An attempt was made to find out whether or not thermostable products of one of the organisms in the presence of the other organism could induce color production. Yeast cultures on whey agar slopes were destroyed by heat, usually 60° to 65°C. for forty-five minutes, and then the mold inoculated but in no instance was there a development of a yellow color. When mold cultures on whey agar slopes were heated and then the yeast inoculated the growth appeared white. Yeast colonies on whey agar plates were also destroyed by heat and molds inoculated but no yellow color developed.

The medium used apparently has considerable influence on the color production by the yeast and mold combination. In a small

number of trials a yellow color was not obtained on synthetic agar, beef extract agar or beef infusion agar while whey agar was repeatedly satisfactory. It is possible that the failure to secure color production with most of the media used was in part due to a poorer growth but it does not seem that this could be the only factor involved.

The mold that was found active in the production of the yellow color was studied with the idea of determining the species. The general characters suggested that it was to be classed as Aspergillus niger. A number of species of Aspergillus were then secured from two laboratories and these tried out for the production of a yellow color in combination with yeast 1. Among cultures of A. flavus, A. niger, and A. terreus from one laboratory and cultures of A. niger, and A. flavus from another, the two cultures of A. niger were the only ones that gave a yellow color in combination with the yeast. These results, which were repeatedly secured, strengthened the belief that the mold isolated was A. niger and showed that the production of the yellow color occurred with cultures of A. niger other than the one isolated but did not occur with certain species belonging to the same genus.

An attempt was made to determine whether or not the production of a yellow color was peculiar to yeast 1 when grown in combination with A. niger or whether it occurred with other yeasts. Color production in colonies on whey agar was observed with a considerable number of yeasts that were grown in combination with mold 1 or with A. niger from other laboratories. A series of cultures belonging to a group of yeasts that produce changes in milk only slowly, if at all, and with which the yeast originally found is to be classed, regularly gave a bright yellow color when grown with A. niger although all gave a white or dirty white growth when grown alone. The lactose fermenting yeasts, T. cremoris and T. sphaerica, also gave a yellow color when grown in combination with the mold. A yellow color was not secured with the yeasts having a pink appearance in pure culture or with certain other types that gave spreading colonies on whey agar. The results show that the production of a yellow color, although it does not occur with all species, is common among

yeasts when they are grown in combination with A. niger, and that accordingly this color production is not likely to be an aid in the classification of the yeasts common in dairy products.

The production of oxalic acid by strains of A. niger[1] and related organisms suggested an attempt to influence the color production of yeasts growing in pure culture by putting a small amount of sterile oxalic acid solution near the colonies. After the development of medium sized colonies on plates, a drop of the sterile acid solution was placed near a number of them and then observations frequently made. No yellow color was secured in any of a considerable number of trials. Attempts were also made using citric acid in place of the oxalic acid, but again only negative results were secured. It seems accordingly that some factor other than the production of oxalic or citric acid by the mold influences the yeast in such a way that it shows a yellow color in the colony. Negative results from the acids would be expected from the fact that when the yeast was grown near killed cultures of A. niger no yellow color developed.

Since the relationship between the molds and yeasts was recognized as a cause of the yellow color production, yellow colonies that were evidently yeasts have been noted on plates a number of times. An examination of such plates has invariably shown the presence of a mold quite like mold 1 and in no instance has a yeast that produces a typical yellow color alone been secured. Frequently the yellow color appeared definitely in a portion of the colony first and then later the colony showed the color throughout.

In one instance a yeast that produced a brownish color in pure culture was isolated. This organism persisted in its color production through a long series of transfers. The color however did not at all resemble the yellow color formed as a result of the combined action of a yeast and mold and there was more danger of its being erroneously classed as a pink than as a yellow.

Aspergillus niger does not seem to be common in dairy products and it may be that its presence in plates poured with these

[1] Aspergillus niger group. Charles Thom and James N. Currie, Jour. Agr. Res., vii, 1, October 2, 1916.

materials is due to air contamination; more than one colony of A. niger on a plate has only rarely been observed.

The development of a decided yellow color by the combined action of a yeast and mold may play a part in the development of a yellow color on certain dairy products such as cheeses, where the conditions for the growth of organisms are very favorable. It seems certain that on some of these materials colored growths occur which cannot be reproduced by a pure culture of any of the organisms present; the fact that associative action of a yeast and mold can yield a yellow color on an agar plate suggests that the same thing may occur where organisms are growing in masses on cheese or some other material.

SUMMARY

The data presented show that A. niger growing near to or in contact with certain yeast colonies on whey agar slopes or plates resulted in the yeast colonies showing an intense yellow instead of the usual white color. This condition occurred with a number of different yeasts common in dairy products, but not with all of those studied. The mechanism of this color production was not worked out, but it is probably rather complex since neither organism growing in contact with killed cultures of the other produced the yellow color.

The species of Aspergillus other than A. niger which were tried did not yield a yellow color when grown with yeasts that did give a yellow color with A. niger.

It seems that the associative action of yeasts and A. niger may be responsible for the yellow color occasionally observed with such products as cheese.

VARIATIONS IN AMOUNT OF MILK AND PERCENT OF FAT IN THE MILK FROM DIFFERENT QUARTERS OF THE COW'S UDDER

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Facts concerning the secretion of milk are becoming of more and more interest to the dairy cattle breeder. Variations in the percentage of fat in the milk are of especial importance. The object of this paper is to present a rather striking fact bearing on this subject, which may with further accumulation of data contribute to more knowledge regarding milk secretion. This data was obtained in connection with some experimental work being done at the Kansas Agricultural College regarding milk secretion.

Previous work in regard to the percentage of fat from the various quarters, was published by Beach in Storrs Report 1904, page 132. In this work only one milking was used and no attempt was made to determine the permanency of the variations. The results did show that when a large number of cows were considered, there was practically no difference in the percent of fat from the various quarters.

In obtaining this data three Jersey cows and two Holsteins were used. The first period lasted two days, or four milkings. Each quarter was milked in a separate pail, four small buckets being used. The milking was done as in common practice, no one quarter being milked dry before the others. The milk from each quarter was weighed, sampled, and tested separately. Two weeks later the exact experiment was duplicated to verify it and test the permanency of the variations.

TABLE 1

NUMBER OF COW	TRIAL	TIME OF MILKING	FRONT UDDER		REAR UDDER		PER CENT OF FAT IN MILK			
							Fore-udder		Rear-udder	
			Right	Left	Right	Left	Right	Left	Right	Left
			pounds	*pounds*	*pounds*	*pounds*	*per cent*	*per cent*	*per cent*	*per cent*
I	1	a.m.	1.9	1.0	3.4	3.1	3.7	3.8	4.55	4.2
		p.m.	2.3	1.3	3.7	2.8	3.85	5.1	4.5	4.0
		a.m.	2.4	1.1	4.0	2.4	4.1	4.4	5.05	4.3
		p.m.	2.1	0.9	3.2	2.1	4.9	4.3	5.2	4.7
	2	a.m.	2.4	1.0	3.5	2.2	4.7	5.4	5.1	4.8
		p.m.	2.4	1.1	3.3	2.3	4.3	4.5	4.8	4.4
		a.m.	2.3	1.1	3.2	2.1	3.8	4.5	4.8	4.3
		p.m.	2.3	1.2	3.3	2.3	4.8	4.8	4.9	4.4
II	1	a.m.	4.5	3.2	7.0	4.7	2.15	2.75	1.85	1.9
		p.m.	5.1	5.1	10.2	7.1	3.3	3.4	3.5	3.5
		a.m.	4.2	3.6	9.0	6.4	3.05	3.5	3.2	3.1
		p.m.	4.4	4.5	9.1	7.0	3.8	4.4	4.3	3.9
	2	a.m.	5.7	5.9	9.3	7.3	3.1	3.8	3.7	3.3
		p.m.	3.8	3.9	7.9	6.4	3.5	3.6	3.2	3.5
		a.m.	4.1	4.4	8.3	6.4	3.2	3.7	3.5	3.7
		p.m.	3.9	5.2	9.3	7.5	3.9	3.6	4.0	3.9
III	1	a.m.	0.9	1.5	1.4	1.4	5.3	5.7	4.2	4.3
		p.m.	0.8	1.5	1.7	1.5	5.25	6.15	6.35	5.9
		a.m.	0.7	1.6	1.5	1.3	5.1	5.75	5.4	5.2
		p.m.	0.7	1.3	1.5	1.4	4.8	5.5	5.7	5.6
	2	a.m.	0.7	1.7	1.8	1.6	5.3	5.8	5.8	5.3
		p.m.	0.7	1.7	1.4	1.3	5.0	5.6	5.5	5.3
		a.m.	0.6	1.4	1.4	1.3	5.7	5.5	7.4	5.4
		p.m.	0.7	1.6	1.6	1.6	5.4	5.8	5.7	5.8
IV	1	a.m.	2.1	2.1	1.2	1.6	6.0	6.1	4.15	4.2
		p.m.	1.8	1.9	1.3	1.9	6.1	6.5	6.8	7.0
		a.m.	1.2	2.0	1.3	1.9	6.0	6.2	5.65	5.7
		p.m.	1.5	1.7	1.0	1.6	5.9	6.0	6.1	6.0
	2	a.m.	2.1	2.0	1.3	2.0	6.2	6.2	5.8	5.9
		p.m.	0.9	1.6	1.2	1.7	6.2	6.3	6.4	6.0
		a.m.	1.5	1.5	1.0	1.5	6.1	6.4	7.0	6.4
		p.m.	1.9	1.8	1.3	1.7	6.2	6.2	6.6	6.0
V	1	a.m.	2.0	3.7	3.6	3.7	4.1	5.5	5.5	4.45
		p.m.	2.0	3.1	3.7	3.5	5.25	6.0	5.95	6.2
		a.m.	1.9	3.5	4.2	3.7	4.45	5.5	5.3	5.05
		p.m.	1.5	2.7	3.1	3.8	4.1	5.0	4.5	4.6
	2	a.m.	1.3	3.0	4.1	3.3	4.3	6.0	5.8	4.6
		p.m.	2.0	2.9	2.4	3.2	4.4	5.8	5.0	5.4
		a.m.	1.2	2.8	3.4	3.2	5.2	6.5	6.5	6.2
		p.m.	1.6	3.4	4.3	3.7	4.2	5.5	5.4	5.2

The figures for both periods are given in table 1. It will be noted the results of both trials check quite closely. Graphs of the milk yeild and the percent of fat of all the cows are given for the first period only.

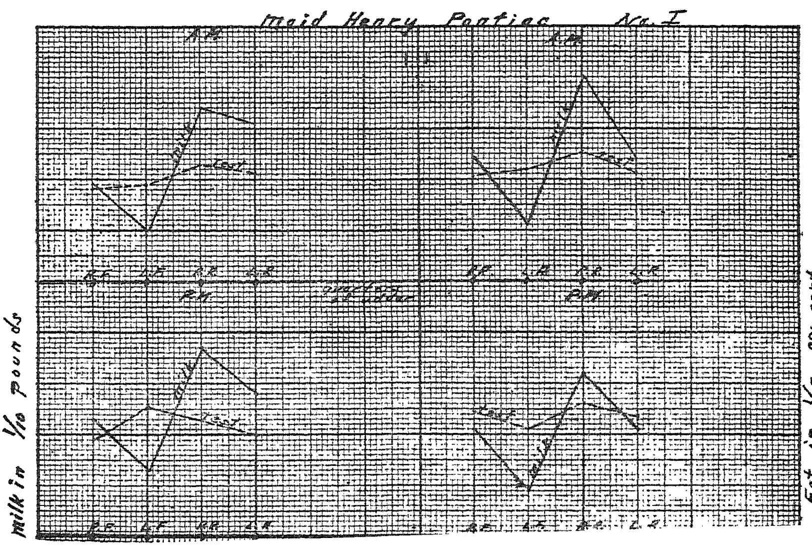
In some cows one certain quarter seemed to presistently test higher or lower in fat then the others. This was especially true of the right fore quarter of cow 5. This quarter was considerably lower in milk than the others.

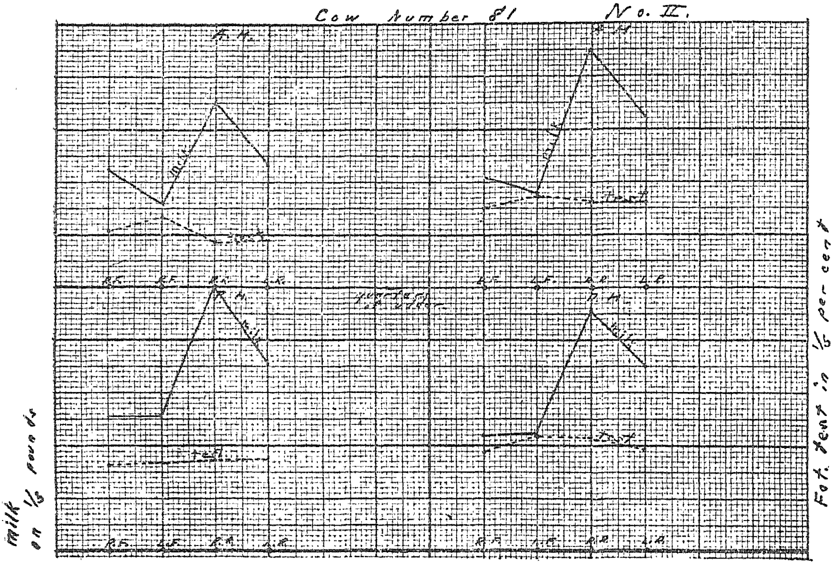
Taking an average of all the cows, no one quarter excelled the others very much either in quantity of milk produced or in per cent of fat. In individuals however certain quarters do appear to vary permanently from the others.

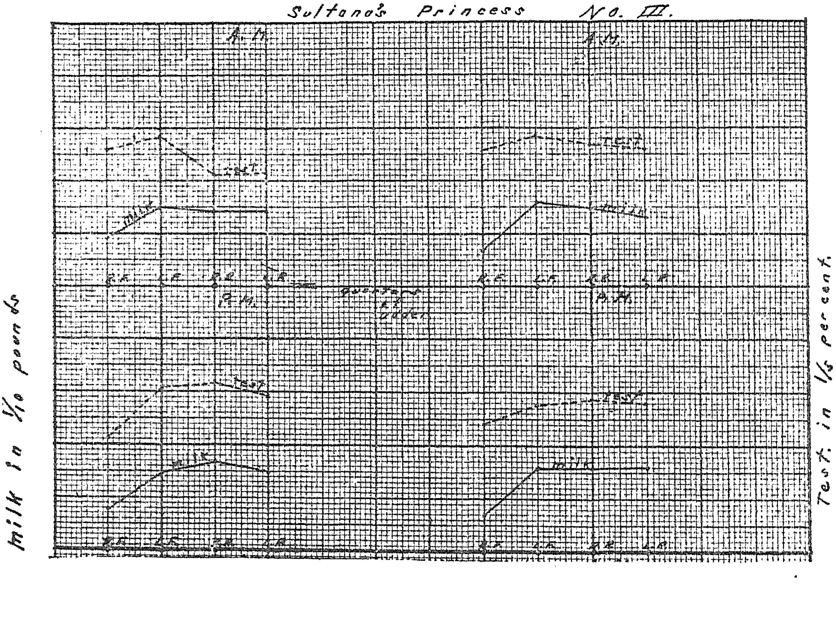
In addition to the cow 5 the right rear quarter of cow number 1 excelled the others in six of the eight tests and this quarter was highest in milk. In cow 3, the right fore quarter was lowest in fat in six of the eight tests and this quarter was much lower than the others in amount of milk produced.

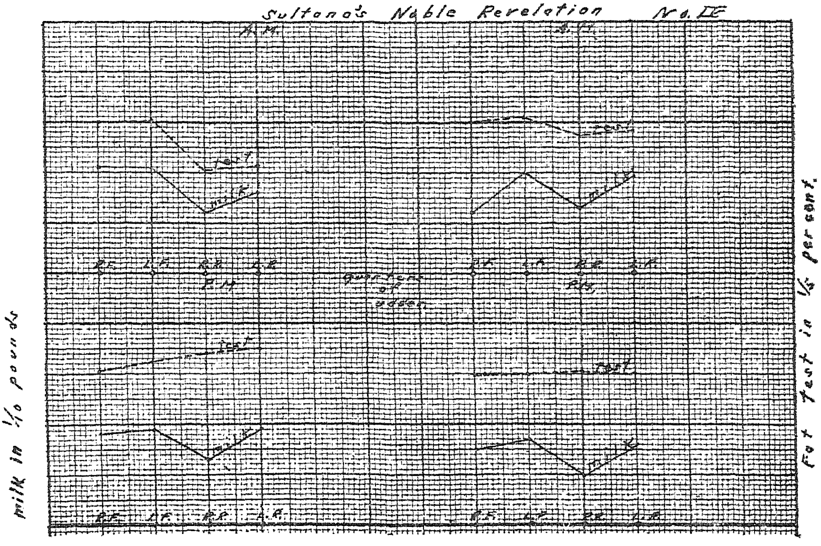
All of the quarters do not vary in fat tests but a few do vary quite noticeably.

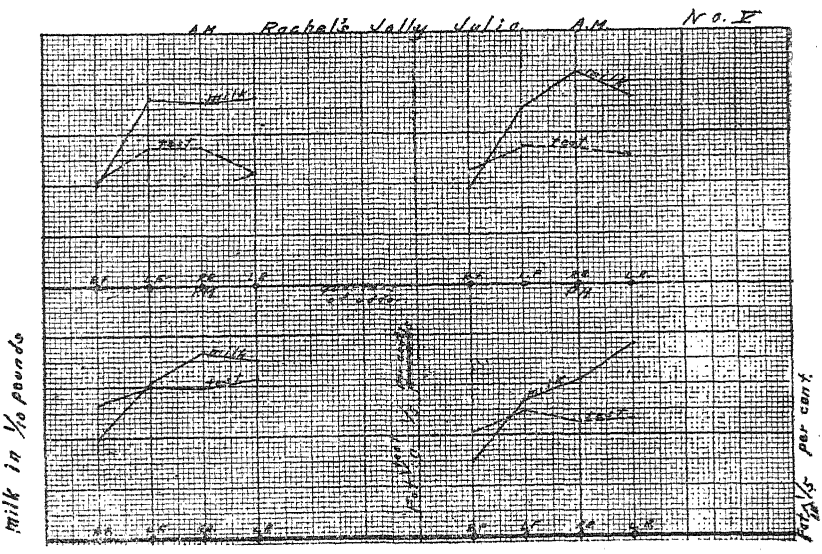
In conclusion, while the fat test for the different quarters was more constant than the milk yields, there appeared to be a tendency for the quarters low in milk to be also low in percentage of butterfat.




Cow Number 81 No. II.
A. M.
P. M.
milk
test
R.F. L.F. R.R. L.R.
quarters of udder
milk in 1/5 pounds
Fat test in 1/5 per cent


Sultano's Princess No. III.
A. M.
P. M.
milk
test
R.F. L.F. R.R. L.R.
quarters of udder
milk in 1/10 pounds
Test in 1/5 per cent.


Sultana's Noble Revelation No. IV
A.M.
test
milk
R.F. L.F. R.R. L.R.
P.M.
Quarters of udder
milk in 1/10 pounds
Fat test in 1/5 per cent.


A.M. Rachel's Jolly Julia A.M. No. V
milk
test
P.M.
milk in 1/10 pounds
per cent.

THE VISCOSITY OF NATURAL AND REMADE MILK[1]

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The viscosity of milk was studied in this Laboratory in connection with an investigation being made of methods of detecting remade or reconstituted milk. This work has already been referred to by Redfield (1) and by one of us (2).

Soxhlet (3) was among the first investigators of this property of milk. He found that the rate of flow varied with the temperature. Woll (4) and Steiner (5) and later Taylor (6), found that heating milk at the pasteurizing temperature decreases the viscosity, while above this temperature the viscosity is increased, Jensen (7) disagrees with the latter statement, claiming that when the albumin is coagulated the viscosity is decreased. According to Bogdan (8) the viscosity is proportional to the total solids but also varies between isotonic samples. Madella (9) determined viscosity at 15°C. and obtained results somewhat higher than those reported in this paper on natural milk at the same temperature. Cavazzani (10) states that the viscosity is different for different cows and for the same cow from day to day. According to Quagliariello (11) and Buglia (12) the viscosity is increased by homogenization. Lucius (13) found that the viscosity rises at the end of lactation and also if the udders are diseased. Oertel (14) maintains that the viscosity does not correspond with the rise or fall in per cent solids and that it varies with the size and nature of the fat globules. Kobler (15), Zangger (16), Polenaar and Fillipo (17), Burri and Nusbaumer (18) and Kooper (19) have also investigated the viscosity. The latter detects watering

[1] Read at the sixty-second meeting of the American Chemical Society in New York City, September 6–10, 1921.

by means of an apparent constant (0.1384) obtained by dividing the viscosity by the total solids.

For the work recorded here the relative viscosity was calculated from the formula;

$$V = \frac{\text{Time of flow of milk} \times \text{specific gravity of milk}}{\text{Time of flow of water} \times \text{specific gravity of water}}$$

The viscosity pipette used is shown in figure 1.[2] The capillary extensions were 10 inches in length. The bulb had a capacity of about 5 cc. and the capillary was of such a size that, using water at 25°C., the pipette drained in about 51 seconds. The water or milk, after adjusting to the desired temperature, was drawn up through the pipette by suction and the time taken for the liquid to flow between points A and B was obtained with a stop watch. The temperature was kept within 0.1° of the desired temperature by circulating water. Variations of as much as 1 second in time of flow occurred in some cases but as a rule duplicate readings within 0.3 second were obtained. The average of three or more readings was taken.

The specific gravity of the milk was calculated from the Quevenne lactometer reading and the per cent total solids was obtained by drying. The acidity of each sample was determined by titration with 0.1 N sodium hydroxide immediately before the viscosity was determined. The peroxidase test was made in two ways as follows: To 10 cc. of milk were added 5 drops of 1 per cent tricresol (20), 5 drops guaiac reagent (4 grams guaiac wood in 50 cc. aceton) and a drop of commercial H_2O_2. To another portion of milk were added 2 drops 1 per cent benzidine in 50 per cent alcohol, 2 drops 1 per cent a-naphthol in 50 per cent alcohol and 1 drop commercial H_2O_2. A blue color develops in the former case and red in the latter, provided the peroxidase has not been destroyed by heat.

Authentic samples of natural milk from herds were obtained and pasteurized at the temperature of about 63°C. for thirty minutes. The milk was then cooled and held at 10–15°C. for

[2] Drawing made by O. S. Rask.

several hours, or over night and used for comparison with remade milk held under the same conditions. Samples of pasteurized market milk were obtained from the local dairies. They were

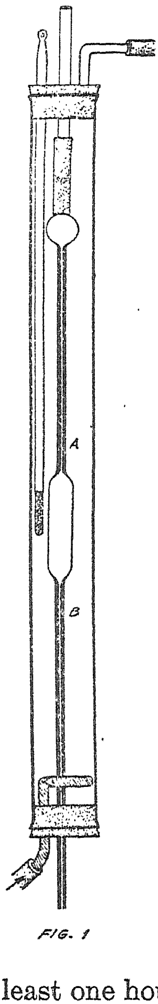

FIG. 1

kept at about 15°C. for at least one hour before determining the viscosity.

Two types of emulsors designated A and B and an homogenizer were used in making the samples of remade milk. In emulsor A

the milk feeds into the bowl from the top and the latter has a speed of 12,000 r.p.m. Emulsor B is what is known as the "suction feed" type, the milk feeding into the bowl from below and the bowl has a considerably higher speed than in the case of emulsor A. The remade milk samples were made from condensed skim milk and unsalted butter, skim milk powder, water and unsalted butter, frozen cream, skim milk powder and water and from whole milk powder, the amounts of the different components being chosen so as to bring the per cent fat and non-fatty solids within the range for natural milk. The milk was pasteurized at about 63°C. for a period of twenty to thirty minutes before it was emulsified. The milk powders were obtained from different manufacturers and were made by several different processes as indicated in tables 3 and 4. In the spray drying process the milk, usually in evaporated form, is sprayed under more or less pressure into a heated chamber, the manner of admitting the hot air and previous treatment of the milk varying with the process used. In the film drying process the milk with or without previous concentration is dried on the surface of one or more steam heated revolving drums. The milk is either sprayed on to the revolving drums or picked up by the latter from below. In some cases the drying cylinders operate under atmospheric pressure and in other cases the cylinders are enclosed in a vacuum chamber and drying is accomplished under reduced pressure.

Superheated condensed skim milk in bulk and frozen cream were obtained from a local ice cream plant and unsalted butter from local distributors.

The temperature at which miik has been held and the length of time it remains at this temperature has considerable effect on the viscosity. A sample of skim milk was pasteurized and divided into two portions. Portion A was kept at 15°C. for one hour and its time of flow through the pipette at 15°C. was 132.7 seconds. Portion B was kept in cold storage for two days at about 3°C. then at 15°C. for one hour and its time of flow was found to be 139.7 seconds. The time of flow as well as the effect of holding whole milk at different temperatures is shown in table 1. A

sample of whole milk was divided into two portions. Portion A was kept at 30°C. for over one hour. Sub-samples were then taken and quickly brought to the desired temperature and the rate of flow was determined at intervals of 5°, from 10° to 40°C. Portion B was kept in cold storage at about 3°C. for one week. Sub-samples of portion B were then treated as in case of portion A. It is seen that the time of flow decreases as the temperature rises and that at 10°C. there is a difference of 13 seconds while at 35°C. there is a difference of only 2.5 seconds in time of flow of two portions of the same sample of milk held at different temperatures.

TABLE 1

Showing difference in time of flow at different temperatures of two portions of the same sample of milk, A held at 30°C. for one hour, B held at 3°C. for one week

	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.	40°C.
A	141.9	122.0	106.7	92.1	82.2	72.4	66.1
B	155.0	132.6	116.6	96.7	84.4	74.9	
Difference, seconds	13.1	10.6	9.9	4.6	2.2	2.5	

TABLE 2

Effect of pasteurization on the viscosity of milk

HEATED 30 MINUTES AT	TIME OF FLOW IN SECONDS AT 20°C.			
	1	2	3	4
35–40°C.	131.6	150.5	129.3	131.0
62–65°C.	130.4	147.8	126.2	129.6
75–80°C.	140.6	154.2		

This change in the viscosity of milk held at different temperatures cannot be attributed to the effect on the fat alone since skim milk also exhibits this phenomenon.[3] The effect of pasteurization on the viscosity of natural milk is shown in table 2. Samples of natural milk were carefully mixed and divided into portions.

[3] While this paper was being prepared for publication a paper on the Chemical and Physical Properties of Remade Milk was published by Palmer and Dahle, Journal of Dairy Science, May 1, 1922, in which results on viscosity are given similar to those reported here.

These portions were heated for thirty minutes at the designated temperatures and any moisture lost by evaporation was replaced with the same weight of distilled water. They were then cooled and held at 17-20°C. for about one hour before the viscosity was determined. It is seen that pasteurization at the ordinary temperature tends to decrease the viscosity slightly. Heating at 75-80°C. for thirty minutes, however, increases the viscosity considerably, this being probably due to a coagulation of the albumin.

A comparison of the viscosity at 25°C. of natural pasteurized milk and remade milk together with mixtures of known amounts of each, is shown in table 3. The relation of viscosity to total solids, the viscosity of water being taken as one, is represented by the expression $\frac{V-1}{T.S.}$ in which V = relative viscosity and T.S. = per cent total solids expressed as parts by weight of milk solids per gram of fluid milk. V–1 represents the viscosity due to the milk solids and is not the same as the relative viscosity of milk. The expression $\frac{V-1}{T.S.}$ gives the same values for skim milk as for whole milk, while the constant of Kooper (21) applies to whole milk only. The samples are arranged in the order of increasing values for $\frac{V-1}{T.S.}$ for remade milk. It is seen that this figure varies between 5.68 and 7.18 for natural pasteurized milk and between 6.26 and 12.60 for remade milk. Figures for the mixtures vary between 5.96 and 8.37. The error in the determination of these figures should not be greater than 0.20. From this table it may be seen that in general there is a certain correlation between the amount of heat to which the milk has been exposed, as indicated by the peroxidase test[4] and the values found for the expression $\frac{V-1}{T.S.}$ in the case of remade milk. As the values for this expression begin to rise somewhat above the maximum value found for natural pasteurized milk, the peroxidase test also

[4] The peroxidase begins to disappear at the temperature of about 72°C.

TABLE 3

Comparison of the viscosity of natural pasteurized milk, remade milk and mixtures of natural and remade milk. Viscosity determined at 25°C.

NATURAL PASTEURIZED MILK				REMADE MILK†					MIXTURES		
Number	Acidity	T.S.	$\frac{\text{V-1}^*}{\text{T.S.}}$	Acidity	Peroxidase test	T.S.	$\frac{\text{V-1}}{\text{T.S.}}$	Remarks‡	T.S.	Percent remade	$\frac{\text{V-1}}{\text{T.S.}}$
1	0.14	11.80	5.68	0.15	Positive	12.65	6.26	Spray skim powder (1) and butter			
2	0.15	12.09	6.16	0.12	Positive	11.76	6.37	Spray skim powder (2) and butter	12.00	20.0	6.21
3	0.12	9.10	6.31	0.12	Positive	8.93	6.44	Spray skim powder (2)	9.08	20.0	6.33
4	0.14	12.20	5.97	0.12	Positive	12.60	6.53	Spray skim powder (1) and butter	12.22	15.0	5.96
5	0.14	12.52	6.34	0.15	Positive	12.48	6.59	Spray skim powder (1) and frozen cream	12.44	23.0	6.38
6	0.14	12.57	6.55	0.14	Positive	12.60	6.59	Spray skim powder (1) and butter	12.65	29.2	6.47
7	0.14	12.41	6.34	0.20	Positive	13.85	6.79	Condensed skim milk and frozen cream	12.60	15.0	6.44
8	0.13	12.23	6.70	0.13	Negative	11.75	6.96	Spray skim powder and butter			
9				0.12	Positive	9.44	7.06	Spray skim powder (1)			
10	0.14	12.60	6.19	0.14	Positive	13.40	7.35	Spray whole milk powder (1). Condensed skim milk and frozen cream	12.52	17.1	6.33
11	0.14	12.40	6.37	0.13	Negative	12.52	7.51	Spray skim powder (1) and butter	12.46	25.0	6.74
12	0.12	9.12	6.21	0.12	Negative	8.96	7.76	Spray skim powder	9.04	25.0	6.21
13	0.14	12.15	6.34	0.14	Negative	12.25	7.84	Spray skim powder (5) and frozen cream	12.33	20.0	6.65
14	0.12	12.25	6.35	0.13	Negative	12.42	8.04	Drum skim powder (10) and butter	12.30	15.0	6.42
15	0.11	9.54	6.99	0.11	Positive	9.02	8.06	Spray skim powder (4)	9.38	25.0	7.39
16	0.14	12.20	6.23	0.14	Trace	12.50	8.16	Spray whole milk powder (1)	12.25	25.0	6.61
17	0.14	12.09	6.20	0.13	Trace	12.09	8.19	Spray whole milk powder (1). (Mixing tank)	12.13	15.0	6.51

18	0.12	12.08	6.03	0.14	Negative	11.84	8.20	Spray skim powder (3) and butter	12.21	30.0	6.84
19	0.12	12.39	6.28	0.14	Negative	11.55	8.22	Spray skim powder (3) and butter	11.99	30.0	6.77
20	0.14	9.65	6.74	0.11		9.00	8.50	Spray skim powder			
21	0.13	12.29	6.10	0.19	Positive	15.02	8.65	Condensed skim milk and butter	12.77	17.3	6.44
22	0.13	13.21	6.96	0.13	Negative	12.15	8.81	Spray skim powder and butter			
23	0.13	13.18	7.18	0.14	Negative	11.80	9.19	Spray skim powder (1) and butter			
24	0.11	13.40	6.71	0.13	Negative	11.75	9.30	Spray skim powder (1) and butter. (Homogenized 49°C. and 3000 pounds pressure)	12.72	30.0	8.37
25	0.12	12.90	6.49	0.13	Negative	12.09	9.33	Spray skim powder (5) and butter			
26	0.12	12.46	6.24	0.14	Negative	11.70	9.46	Spray skim powder and butter	12.26	30.0	7.28
27	0.12	12.06	6.22	0.12	Positive	12.55	9.47	Spray whole milk powder (1)	12.16	20.0	6.96
28	0.12	9.16	6.09	0.10	Negative	8.85	9.54	Drum skim powder (9)	9.13	15.0	6.57
29	0.14	11.40	6.44	0.14	Negative	11.30	10.01	Spray skim powder and butter	12.30	20.0	7.07
30	0.12	12.90	6.95	0.07	Negative	12.04	11.83	Drum whole milk powder (11)	12.73	15.0	7.50
31	0.14	12.54	6.14	0.05	Negative	12.59	12.23	Drum skim powder (11) and frozen cream	12.52	20.0	6.95
32	0.20	12.29	6.36	0.05	Negative	12.55	12.60	Drum skim powder (11) and frozen cream	12.44	25.0	7.16

* The viscosity may be calculated from this expression as follows: For natural pasteurized milk No. 1 T.S. = 11.80 and $\frac{V-1}{T.S.} = 5.68$. Then $\frac{V-1}{0.1180} = 5.68$ or V-1 = 0.67 or V = 1.67.

† The samples numbered 7, 11, 13, 16, 21, 31 were made with emulsor B. The remaining samples were made with emulsor A, unless otherwise indicated. The authors are indebted to Dr. H. W. Redfield for his assistance in preparing a number of these samples.

‡ The spray powders were made by two different processes. In case of one process different numbers in parenthesis indicate different manufacturers. In case no number is given, the spray powder was made by a different process, U. S. Patents 1,078,848; 1,107,784; 1,157,935; 1,266,013. In the case of drum powders different numbers in parenthesis indicate different processes.

TABLE 4

Comparison of the viscosity of natural pasteurized milk and remade milk. Viscosity determined at 15°C.

NATURAL PASTEURIZED MILK*				REMADE MILK†					REMARKS
Number	Acidity	T.S.	$\frac{V-1}{T.S.}$	Number	Acidity	Peroxidase test	T.S.	$\frac{V-1}{T.S.}$	
33	0.13	12.50	7.35	33	0.14	Positive	12.25	7.13	Spray skim powder (7) and butter
36	0.13	12.13	7.42	34	0.13	Positive	12.18	7.43	Spray skim powder (6) and butter
38	0.13	12.42	7.67	35	0.16	Positive	12.98	8.06	Spray skim powder (1) and butter
41	0.13	12.20	7.39	36	0.12	Positive	12.50	8.34	Spray skim powder (1) and butter
42	0.13	12.42	7.26	37	0.12	Positive	9.18	8.44	Condensed skim milk
44	0.13	11.86	7.35	38	0.13	Positive	12.27	8.76	Spray skim powder (4) and butter. (Homogenized at 44°C. and 2500 pounds pressure)
45	0.15	12.14	7.20	39	0.13	Positive	12.90	8.92	Spray whole milk powder (1). (Homogenized at 50°C. and 2500 pounds pressure)
46	0.12	13.11	7.32	40	0.14	Positive	9.24	9.17	Dough process skim powder. (Mixing tank)
MARKET MILK				41	0.11	Positive	12.40	9.19	Spray skim powder (1) and butter. (Homogenized at 48°C. and 2500 pounds pressure)
60	0.13	11.97	6.78	42	0.15	Positive	12.50	9.38	Spray whole milk powder (1)
61	0.14	10.50	7.03	43	0.16	Trace	11.98	9.52	Condensed skim milk and butter
62	0.14	12.56	7.43	44	0.12		12.06	9.65	Spray skim powder and butter
63	0.14	12.36	6.96	45	0.08	Positive	11.85	10.26	Drum process skim powder (11) and butter
64	0.16	12.48	6.70	46	0.13	Negative	12.10	11.70	Spray skim powder (8) and butter
65	0.14	12.05	6.78	47	0.09	Negative	11.95	13.90	Drum whole milk powder (11)

66	0.13	12.75	7.11	48	0.07	Negative	12.13	16.07	Drum whole milk powder (12)
67	0.13	12.05	7.11	49	0.11	Negative	12 48	16 63	Drum whole milk powder (13)
68	0.14	12.11	6.99						
69	0.14	12.60	7.36						
70	0.17	11.97	7.18						
71	0.14	12.05	6.95						
72	0.14	12.25	7.36						
73	0.14	12.30	7.32‡						
74	0.14	11.68	7.69						
75	0.14	12.15	7.40						
76	0.15	11.95	6.89						
77	0.13	12.14	7.40						
78	0.15	13.26	7.63‡						
79	0.14	12.68	8.06‡						

* These samples were strained through cheese cloth. The market milk samples were not strained.

† Emulsor A was used in making these samples unless otherwise indicated. In the case of spray powders, different manufacturers using the same general process are indicated by different numbers in parenthesis. In case no number is given (no. 44) the powder was made by a different process. See last note table 3. In case of drum powders different numbers in parenthesis indicate different processes.

‡ Raw milk.

becomes negative. However in the case of no. 8 a negative peroxidase test is accompanied by a low viscosity. No reason can be assigned for this discrepancy nor for the high viscosity accompanying a positive peroxidase test in the case of sample 15. In respect to samples 16, 17 and 27 the high values for $\frac{V-1}{T.S.}$ are to some extent due to the homogenizing effect on the fat of the high pressure used in the process of manufacture of the powder. The results obtained for the mixtures given in this table, with the exception of no. 24, differ to a small extent only from those obtained for natural pasteurized milk, the difference being either within the range of experimental error or within the range of variation found for natural milk. It is apparent therefore that a determination of the viscosity would be of little use in detecting mixtures of natural pasteurized milk and remade milk in proportions such as recorded here. In the samples of remade milk shown in this table for which the values for $\frac{V-1}{T.S.}$ are within the range found for natural pasteurized milk, the colloidal condition of the powder has apparently not been changed in the process of manufacture in so far as it affects the viscosity. The high viscosity shown by remade milk made from some drum and spray powders is apparently due to the high heat to which the powders have been exposed in the process of manufacture which tends to coagulate the albumin and make the powder dissolve with more or less difficulty.

In table 4 a comparison is shown of the viscosity figures obtained at 15°C. for natural and remade milk. Here similar relations are shown as in table 3, the values for $\frac{V-1}{T.S.}$ however being higher due to the lower temperature used in determining the viscosity. As in table 3 the high viscosity shown by remade milk samples 38, 39, 41 and 42 is partly due to homogenization while in the case of samples 45, 47 and 48 the abnormally low acidities probably to some extent influence the viscosity. The positive peroxidase test given by samples 40 and 45 indicates that the powders used have not been exposed to a high degree

of heat. A determination of the albumin in these two samples showed that little or no coagulation had taken place.

TABLE 5

Showing effect of the emulsor and homogenizer on the viscosity of natural and remade milk

Viscosity determined at 25°C.

NUMBER		$\frac{V-1}{T.S.}$
	Natural pasteurized milk	
50	Natural pasteurized milk	7.09
50	Above homogenized at 43.3°C. and 3500 pounds pressure	10.64
56	Natural pasteurized cream*	9.22
56	Above homogenized at 43.3°C. and 1200 pounds pressure	49.10
54	Natural pasteurized milk	6.84
54	Above homogenized at 43.3°C. and 1200 pounds pressure	7.09
55	Natural pasteurized milk	6.30
55	Above emulsified, emulsor A	7.17
26	Natural pasteurized milk	6.24
26	Above emulsified, emulsor B	6.46
2	Natural pasteurized milk	5.94
2	Above emulsified, emulsor B	5.86
	Remade milk†	
50	Condensed skim milk and butter, homogenized at 41.6°C. and 3500 pounds pressure	16.65
51	Condensed skim milk, frozen cream homogenized at 43.3°C. and 3500 pounds pressure	14.95
21	Condensed skim milk and butter, emulsor A	8.65
7	Condensed skim milk and frozen cream emulsor A	6.79
52	Spray skim powder (5) and butter, homogenized at 43.3°C. and 1200 pounds of pressure	8.18
53	Spray skim powder (5) frozen cream, homogenized at 42.7°C. and 1200 pounds pressure	9.56
5	Spray skim powder (1) and frozen cream emulsor B	6.59
6	Spray skim powder (1) and butter, emulsor B	6.59
54	Drum skim powder (11) and butter, homogenized at 43.3°C. and 1200 pounds pressure	13.51
55	Drum skim powder (11) and butter emulsor A	12.95
31	Drum skim powder (11) and frozen cream, emulsor A	12.23
32	Drum skim powder (11) and frozen cream, emulsor B	12.60

* 19.24 per cent fat.

† Figures in parenthesis indicate different manufacturers.

The effect of the emulsors and homogenizer on natural pasteurized milk and remade milk is shown in table 5. Homogenization at 3500 pounds pressure increases considerably the viscosity of milk as well as cream. A pressure of 1200 pounds also increases the viscosity of cream considerably, but seemed to increase the viscosity of milk to a small extent only. The emulsor increases the viscosity of milk slightly or not at all.

The effect of putting natural and remade milk through a high speed centrifuge[5] (30,000 r.p.m.) on the relation of viscosity to

TABLE 6

Effect of high speed centrifuge on relation of viscosity to total solids

NUMBER	ORIGINAL	SKIMMED ONCE	SKIMMED TWICE	SLIME FROM BOWL
	$\frac{V-1}{T.S.}$ on natural pasteurized milk			
11	6.37	6.13	5.21	6.07
32	6.36	5.81	4.82	6.07
5	6.34	6.39	5.66	8.53
6	6.55	6.47	5.55	7.94
	$\frac{V-1}{T.S.}$ on remade milk			
11	7.51	7.98	5.56	
32	12.60	12.94	9.06	
5	6.59	6.51	4.92	12.66
6	6.59	5.28		

* The composition of these samples is given in table 3.

total solids, $\frac{V-1}{T.S.}$ is shown in table 6. Column 2 in this table, shows the values obtained on the original milk. This milk was then run through the centrifuge and the values obtained on the skim milk, which are shown in column 3. This skim milk was then run through the centrifuge again. The figures obtained on the second skim milk are given in column 4. From table 6 it may be seen that there is practically no change in the relation

[5] This centrifuge differs from a cream separator mainly in the speed of the bowl.

of viscosity to the solids when milk is skimmed once, but the second skimming appears to change the composition of the skim milk by removing milk solids so that the values for $\frac{V-1}{T.S.}$ are lowered. This is more marked in the case of remade milk. Considerable difference was noted in the sediment left in the bowl of the centrifuge. The sediment from the natural milk was easily mixed with water and had a viscosity only slightly greater than that of the original milk. The sediment from the remade milk, however, besides being larger in amount, was packed against the side of the bowl in a hard, bony mass and in one case only could the sediment be mixed with water and its viscosity determined.

SUMMARY

The relation of viscosity to the total solids is shown by means of the expression $\frac{V-1}{T.S.}$ in which

$$V = \frac{\text{Time of flow of milk} \times \text{specific gravity of milk}}{\text{Time of flow of water} \times \text{specific gravity of water}}$$

and T.S. = total solids expressed as parts by weight of milk solids per gram of fluid milk. For a given number of samples the values for $\frac{V-1}{T.S.}$ for natural pasteurized milk varied from 5.68 to 7.18 and for remade milk from 6.26 to 12.60 at 25°C. The figures for mixtures containing 15 to 30 per cent of remade milk varied from 5.96 to 8.37. When the viscosity was determined at 15°C. the figures varied from 7.20 to 7.67 for natural pasteurized milk, from 6.70 to 8.06 for market milk and from 7.13 to 16.63 for remade milk.

The viscosity of milk as determined is, to a certain extent, dependent upon the temperature at which the milk has been held.

The viscosity is decreased slightly by pasteurization at 62–65°C. for 30 minutes, but considerably increased by heating at 75–80°C. for the same length of time.

Homogenization at a high pressure has considerable effect on the viscosity, while the emulsor has little or no effect.

By means of a high speed centrifuge it was found possible to throw out milk solids from both natural and remade milk to such an extent as to lower the values for $\frac{V-1}{T.S.}$ as compared with the values for the original milk. The sediment obtained from remade milk differed in quantity and character from that of natural milk.

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THE RELATION BETWEEN AGE AND FAT PRODUCTION IN DAIRY COWS

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Milk secretion, like other physiological processes changes with age in a definite way. It was observed at an early date that milk and fat production, on the average gradually increases as the dairy cow becomes mature and then gradually decreases with the onset of old age; thus under similar conditions of feeding and management a heifer is expected to increase her yearly production at each succeeding lactation period until she reaches maturity.

There has been, however, more or less uncertainly among breeders of dairy cattle as to the age of maturity and maximum milk and fat production. The rate of increase of production with each succeeding year and lactation is also a debated question.

At the time of the adoption of the advanced registry system by the several dairy cattle breed associations, accurate data were not available and therefore arbitrary minimum requirements for entrance varying from 250.5 pounds of fat at two years to 360 pounds of fat at five years were established. While these requirements were arbitrary and not based on data, they have been widely accepted by dairymen as indicating that milk secretion increases in a linear manner and that maximum production was reached by the time the dairy cow was five years old.

Since the inauguration of the advanced registry system in this country many thousands of yearly and seven-day records have been completed and reported in the herd books of the several breed associations. The records furnish excellent data to determine the relation between age and milk secretion throughout the entire productive life of the dairy cow.

Pearl and co-workers (1) at the Maine Station made use of a limited number of records in showing that the curve relating milk flow to age in dairy cattle was generally of logarithmic form. Hooper (2) of the Kentucky Station and McCandlish (3) of the Iowa Station have also presented a limited amount of data showing the relation between age and production.

The data presented by these workers being rather limited, it was thought worth while to compile all the available data. With the coöperation of the advanced registry departments of the several breed associations, it has been possible to secure practically all the fat production records made in this country previous to the recent changes in the minimum requirements.

In making a study of these records, it was realized that the records are of a selected population. In the first place the minimum entrance requirements eliminate all animals incapable of meeting this standard. In the second place, the cows of advanced ages are also more or less selected, as only cows of exceptional ability as producers would be tested at advanced ages, and due to the declining productive ability as they grow old they enter the advanced registry with increasing difficulty. These shortcomings must be kept in mind in interpreting and in making use of these data.

In table 1 are presented the results of a study of over 46,000 yearly records and over 104,000 seven-day records classified by age intervals of one year. As will be seen from figure 1 the age of maximum production varies but slightly between the several breeds and does not appear to be significant. It will be seen that fat production gradually increases up to between seven and eight years of age on the average, and then gradually decreases with the onset of old age. The minimum requirements are also plotted, beings raised 100 pounds for convenience of comparison. It will be seen from the difference in the slope of the two curves that up to eight or nine years of age the older the cow the easier it is for her to exceed the minimum requirements, but that after that age animals are at a constantly increased handicap due to the effect of old age on production.

In figure 2 the weighted average production of all breeds on yearly test is plotted in terms of the percentage of the maximum production. By means of this chart one can easily determine what may be expected of a dairy cow at various ages in relation to her maximum production. In a previous paper (4) the course

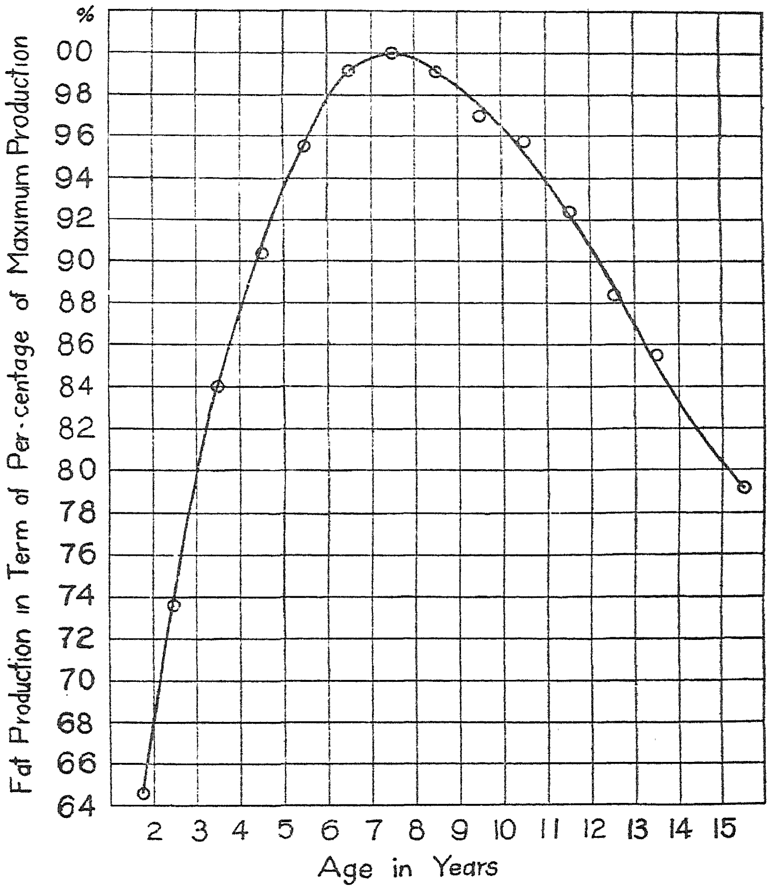

FIG. 2. THE RELATION BETWEEN AGE AND FAT PRODUCTION EXPRESSED AS PERCENTAGE OF MAXIMUM PRODUCTION

This curve represents over 45,000 yearly records including practically all official records of the Ayrshire, Guernsey, Holstein, Jersey, and milking Shorthorn breeds.

of growth with age on lactating Jerseys was presented. It was shown that the Jersey cow increases in body weight with age up to about eight years. A comparison of these data (fig. 3) shows that growth in weight and increase in milk secretion of the Jersey cow follows the same course up to the time of maturity. It is inferred from this close relation that the upward trend of the milk secretion curve with age is due to growth and the accompanying physiological changes.

After about eight or nine years of age milk secretion gradually declines. The declining curve of milk secretion, in the absence of contradictory evidence, is then thought to be a measure of the rate of growing old (senescence) and of the physiological changes accompanying advancing age, just as the rising segment of the curve of milk secretion was found to be a good measure of the rate of growth (fig. 3). The milk secretion at different ages may be said to be the resultant of two physiological processes, growth and senescence; from the age milk secretion begins to about eight or nine years, the process of growth predominates with a consequent increase in milk secretion, while after that age the process of senescence predominates with a consequent decrease in milk secretion.[1]

From the standpoint of dairy cattle breeding, this data is also of great value as it makes possible the comparison of milk production records made at any age. In order to make studies of the transmission of milk secretion by the dairy sires as well as by the dams, it becomes necessary to convert all records of production to a comparable basis. It is suggested that a convenient method is to convert records made at any age to their "mature equivalent" by means of this data.

A record of production made at any age can be converted to the "mature equivalent" by multiplying the actual production made at that age by the ratio of the average production at maturity (see table 1 and fig. 1) to the average production at the age the record in question was made.

The following example will illustrate the method. If a cow at four and one-half years produces 624 pounds of fat, what is

[1] Certain theoretical considerations of this problem are presented in the Jour. of Gen. Physiol. vol. vi, September, 1923.

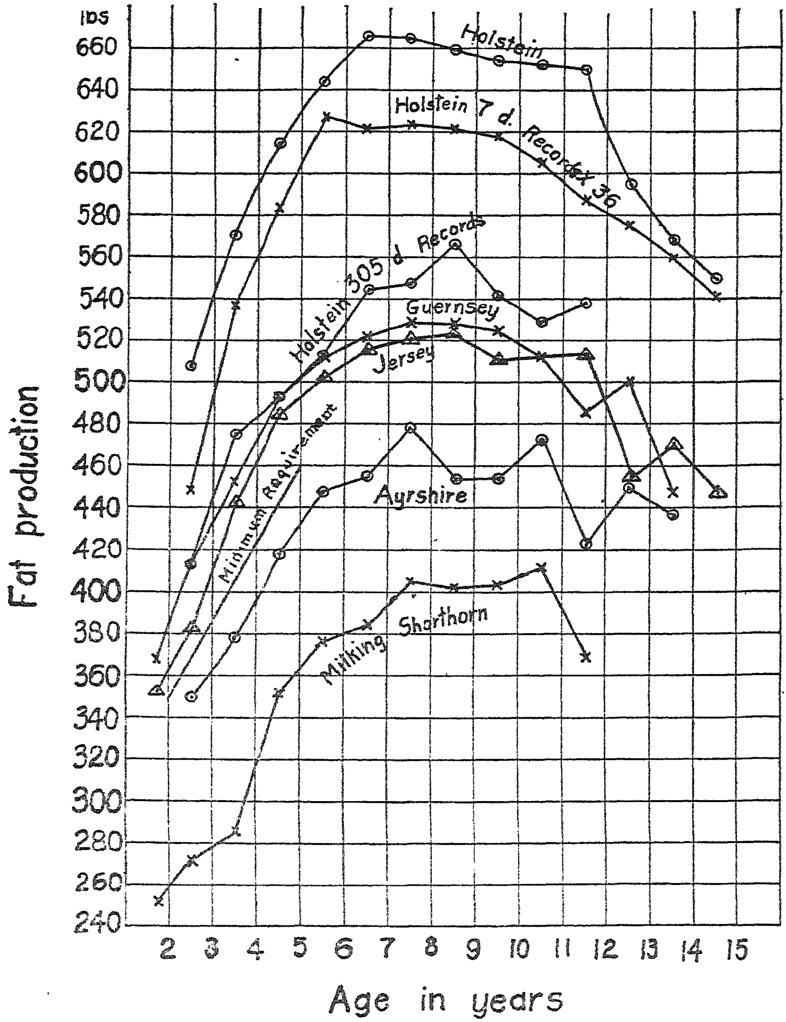

FIG. 1. THE RELATION BETWEEN AGE AND FAT PRODUCTION IN DAIRY CATTLE

With the exceptions noted the curves represent the butterfat production on yearly test. The minimum requirements are raised 100 pounds for convenience of comparison.

TABLE 1

The relation between age and yearly feed production of dairy cattle

AGE	AYRSHIRE COWS		GUERNSEY COWS		HOLSTEIN COWS				JERSEY COWS		MILKING SHORTHORN COWS		WEIGHTED AVERAGE OF ALL COWS		FAT EXPRESSED AS PER CENT OF MAXIMUM PRODUCTION	SEVEN DAY RECORDS	
					365 day records		305 day records										
	Number of cows included	Fat per year	Number of cows included	Fat per year	Number of cows included	Fat, 365 days	Number of cows included	Fat, 305 days	Number of cows included	Fat, 365 days	Number of cows included	Fat per year	Number of cows included	Average yearly fat production		Number of animals	Pounds of fat
years		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*			
1.7			313	368					947	353	15	252	1,275	355.4	64.6	33,765	12.46
2.5	1,710	350	5,241	412	2,454	508	1,250	413	4,090	383	306	272	15,001	404.9	73.6	22,019	14.91
3.5	903	378	2,566	452	1,523	570	762	475	2,263	443	167	285	8,184	462.0	84.0	16,374	16.20
4.5	716	418	1,977	492	1,238	615	606	493	1,687	486	125	352	6,349	497.1	90.4	11,259	17.39
5.5	545	448	1,133	511	1,116	644	467	513	1,487	502	75	376	4,823	424.7	95.5	8,356	17.26
6.5	399	455	897	521	835	666	331	544	1,067	516	80	383	3,609	544.8	99.1	5,586	17.32
7.5	298	478	572	528	583	665	223	547	837	521	66	405	2,579	549.4	100.0	3,256	17.25
8.5	225	453	369	527	396	659	156	566	565	524	65	402	1,776	544.9	99.1	1,862	17.19
9.5	155	454	264	524	232	654	72	541	355	510	43	403	1,121	533.2	97.0	1,054	16.80
10.5	100	472	123	512	111	652	46	529	200	509	29	411	609	526.4	95.8	548	16.34
11.5	52	423	76	486	59	650	17	538	108	513	21	369	333	507.9	92.4	285	15.96
12.5	26	450	32	500	37	595	13	441	58	453	13	397	179	485.3	88.3	130	15.54
13.5	15	436	24	447	11	569	6	510	31	470	4	399	91	469.8	85.5	42	15.04
14.5	8	375	5	491	4	550			13	447	3	360	33	440.7	80.2	24	15.41
15.5	4	392	4	446	4	475			8	451	2	353	22	434.8	79.1	16	15.43
16.5	2	458	1	395	2	500			5	465			10	463.6		4	18.37
17.5	2	376	2	410					1	590			5	432.4		3	15.50
18.5	1	411							1	413			2	412.0			
19.5	0												0				
20.5	1	375											1	375.0			
	5,162		13,599		8,605		3,949		13,723		1,014		46,002			104,583	

* Compiled from the records of *Register of Merit* Jersey, *Record of Merit* Shorthorn, *Advanced Register* Ayshire, Guernsey and Holstein cattle.

her "mature equivalent" record? The weighted average fat production of all breeds at four and one-half years is 497.1 pounds of fat and at maturity, 549.4 pounds of fat (see table 1) therefore:

$$624 \times \frac{549.4}{497.1} = 689.6 \text{ pounds of fat (mature equivalent).}$$

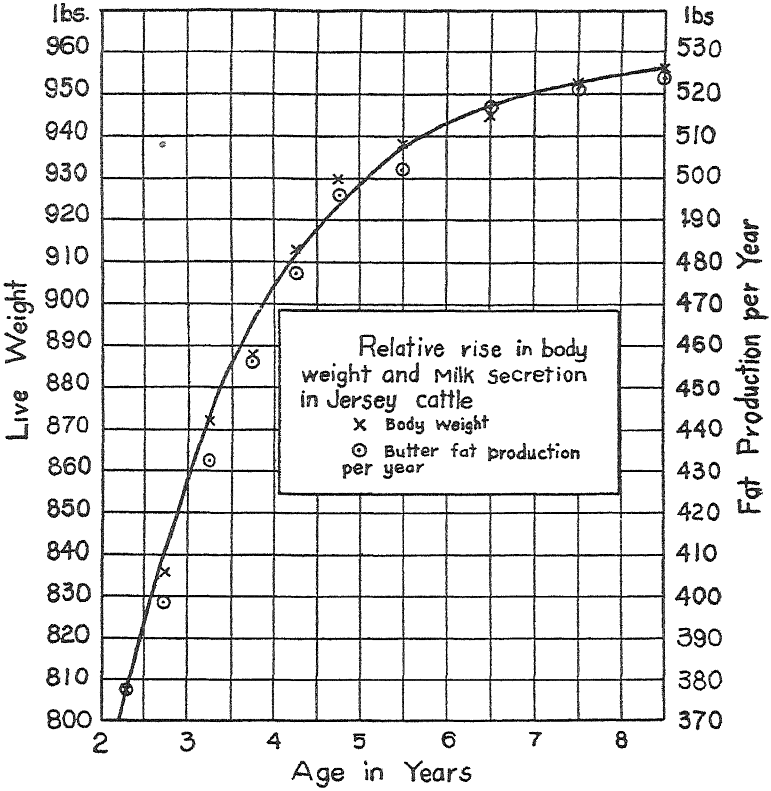

FIG. 3. RELATIVE INCREASE IN WEIGHT AND FAT PRODUCTION IN JERSEY CATTLE

The increase of body weight with age is indicated by the crosses and the fat production indicated by the circles. It will be noted that both follow the same course.

In other words, a cow capable of producing 624 pounds of fat at four and one-half years of age, under similar conditions of feeding and management may be expected to produce 689.6 pounds of fat at maturity.

The records of production of each of the dairy breeds may be converted by this method, using the data of that particular breed.

Studies on the transmitting ability of the sires of each of the dairy breeds, using the above method, are now in progress.

SUMMARY

Data was presented showing the relation between age and yearly production of over 46,000 cows of the Jersey, Guernsey, Holstein-Friesian, Ayrshire and Dairy Shorthorn breeds and over 104,000 Holstein-Friesian seven-day records.

It was shown that fat production gradually increases up to between seven and eight years of age on the average, and then gradually decreases with the onset of old age.

No significant difference between the breeds in the time of maturity was noted.

The close relation between an increase in body weight and milk secretion with age is interpreted as indicating that the upward trend of the milk secretion curve with age is due to growth while the descending segment of the curve is due to senescence or the physiological changes due to old age.

A method of converting the fat production records of dairy cows made at any age to the "mature equivalent" was presented as an aid in making studies of the transmitting ability of dairy sires.

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THE EFFECT OF MILK PLANT OPERATIONS ON THE AMOUNT OF CREAM RISING ON MILK[1]

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The consumer of milk is inclined to judge its richness by the volume of cream appearing in the top of the bottle. Unfortunately, this basis of measurement is not a reliable indication of the amount of butter fat present in milk. It is entirely possible for milk to contain the legal fat requirement and yet show no cream in the bottle. Seldom is this true, however, upon milk which is produced and bottled on the farm, but when milk is transported to the milk plant and subjected to the various treatments given it there, it more often reaches the consumer showing a reduced cream layer in the bottle.

A study of the effect of milk plant operations on the creaming ability of milk was made at the Pennsylvania State College Creamery. The factors studied included clarification, pumping cold and hot milk, agitating hot milk, and different methods of heating and cooling pasteurized milk.

The creaming ability of the milk was measured by taking duplicate samples before and after each operation, in cylinders graduated to read the per cent by volume of cream. These cylinders were held at ice water temperature (35°F.) and hourly readings were taken for twenty-four hours. Unless otherwise stated the last readings are used in making a summary of the results.

I. THE EFFECT OF CLARIFICATION

Milk at 55°F. was clarified by passing it through a DeLaval centrifugal clarifier. Samples of milk were taken before and

[1] Submitted by W. H. Martin in partial fulfillment of the requirements for the degree of Master of Science at the Pennsylvania State College.

after clarification for cream line readings. Similar tests were made with milk clarified at 90°F., and with milk clarified at 55°F. and heated at 90°F. after clarification.

TABLE 1

TRIAL	PER CENT VOLUME OF CREAM ON					
	Unclarified	Clarified 50°F.	Unclarified	Clarified 90°F.	Unclarified	Clarified, heated to 90°F.
1	15	14	15	15	17	17
2	17	16	14	14	16	16
3	16	14	17	16	15	15
4	20	15	16	17	15	14
5	17	15				
6	19	16				
7	18	16				
8	19	16				
Average......	17.6	15.25	15.5	15.5	15.75	15.5

II. EFFECT OF PUMPING HOT AND COLD MILK

Cold milk was pumped from the clarifier to the pasteurizer. Samples were taken before clarification, after clarification and from the discharge of the pump. Trials were also run in which milk was pasteurized, and half of it was pumped while hot over a cooler, where it was cooled to 50°F.; the other half was allowed to flow by gravity over the cooler. Samples were taken of the

TABLE 2

TRIAL	PER CENT VOLUME OF CREAM RISING ON MILK		
	Unpumped raw	Unpumped clarified	Clarified and pumped
1	15	14	15
2	17	16	16
3	16	14	13
4	17	15	17
5	17	15	17
6	17	16	16
7	17	15	16
Average............	16.57	15	15.71

milk before pasteurization and of the pumped and unpumped pasteurized milk after it was cooled.

TABLE 3

TRIAL	PER CENT VOLUME OF CREAM RISING ON MILK		
	Raw	Pasteurized and pumped	Pasteurized not pumped
1	17.0	17.0	17.0
2	16.0	15.0	16.0
3	16.0	13.5	15.0
4	16.0	15.5	16.5
5	16.0	16.0	16.5
6	17.0	17.0	17.0
7	17.0	17.0	18.0
Average............	16.4	15.7	16.6

III. LENGTH OF TIME OF HOLDING IN THE PASTEURIZER WITH AGITATION

In market milk plants it is often necessary to hold the pasteurized milk for some time before it is cooled. A study was made of this factor in order to determine how long the milk could remain in the pasteurizer while hot and subject to agitation, without affecting the creaming ability of milk.

Observations were made on milk held in the pasteurizer while hot for different periods of time. Samples were taken hourly and the volume of cream that came to the surface was recorded The results of four trials are indicated below by means of the chart.

IV. EFFECT OF PASTEURIZATION AND METHOD OF COOLING ON CREAM LINE OF MILK

Trials were made in which milk was pasteurized and part was cooled in the vat, and part cooled quickly by passing the milk over a surface cooler as soon as the holding period was over. A comparison was made with other pasteurizers by pasteurizing part of the milk in each machine. The results of these trials are recorded in the following table.

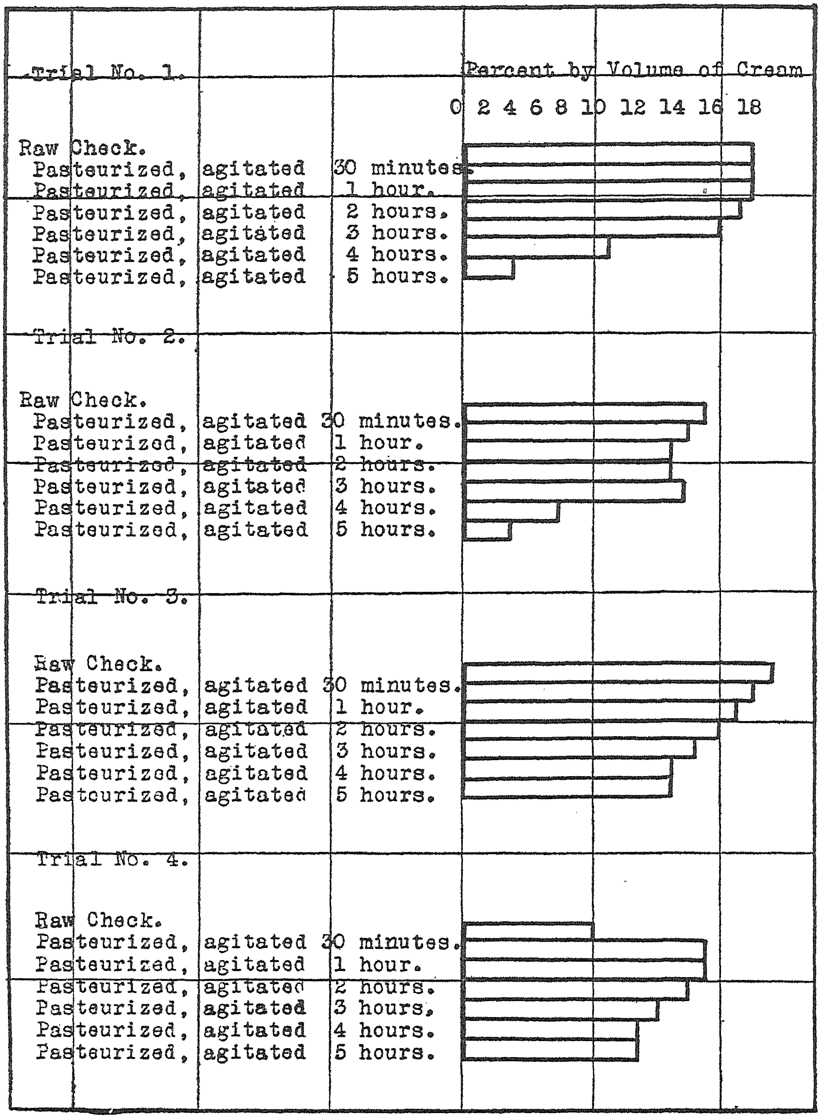

Trial No. 1.
Percent by Volume of Cream
0 2 4 6 8 10 12 14 16 18
Raw Check.
Pasteurized, agitated 30 minutes.
Pasteurized, agitated 1 hour.
Pasteurized, agitated 2 hours.
Pasteurized, agitated 3 hours.
Pasteurized, agitated 4 hours.
Pasteurized, agitated 5 hours.
Trial No. 2.
Raw Check.
Pasteurized, agitated 30 minutes.
Pasteurized, agitated 1 hour.
Pasteurized, agitated 2 hours.
Pasteurized, agitated 3 hours.
Pasteurized, agitated 4 hours.
Pasteurized, agitated 5 hours.
Trial No. 3.
Raw Check.
Pasteurized, agitated 30 minutes.
Pasteurized, agitated 1 hour.
Pasteurized, agitated 2 hours.
Pasteurized, agitated 3 hours.
Pasteurized, agitated 4 hours.
Pasteurized, agitated 5 hours.
Trial No. 4.
Raw Check.
Pasteurized, agitated 30 minutes.
Pasteurized, agitated 1 hour.
Pasteurized, agitated 2 hours.
Pasteurized, agitated 3 hours.
Pasteurized, agitated 4 hours.
Pasteurized, agitated 5 hours.

Chart 1

The results obtained in this experiment showed that there is a reduction of 30, 38, and 60 per cent in the volume of cream which formed on milk pasteurized and cooled in the glass lined vat as compared with the volume of cream rising on raw milk. The loss amounted to 7, 8 and 15 per cent, when the cooling was done by passing the milk over a cooler as soon as the holding period was over. When the same milk was pasteurized and cooled in a pasteurizer of the coil vat type the loss was 8, 14 and 15 per cent; when cooled over a cooler the loss was 3 and 7 per cent in two trials and a gain of 8 per cent in one trial.

TABLE 4

	PASTEURIZER					
	Coil vat			Glass lined vat		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Temperature of raw milk °F......	65	68	68	65	68	68
Time in minutes required to heat to 142°F......................	32	23	43	18	17	22
Time in minutes held at 142°F....	25	25	25	25	25	25
Time in minutes to cool to 50°F..	55	90	75	130	120	110
Per cent fat.....................	3.8	3.7	3.9	3.8	3.7	3.9
Per cent by volume of cream rising on raw milk...............	12	13	14	12	13	13
Per cent of cream rising on milk cooled quickly over cooler......	13	13½	13	11	11	12
Per cent of cream rising on milk cooled slowly in vat...........	11	11	12	8	5½	9

The method of cooling milk and the agitation it is subjected to during pasteurization, holding and cooling seems to play an important part in the creaming ability of milk. When cooling was done in the vat, the cream line was materially effected. The amount of decrease was influenced by the amount and kind of agitation to which the milk was subjected. This loss can be over come by cooling the milk quickly without agitation.

It is the common belief that the creaming ability of milk is affected by some mechanical effect upon the fat globules themselves. It is generally believed that by breaking up the fat globules a reduction in cream layer results. The results submitted

in this paper showing the effect of agitating hot milk upon the cream layer of milk might indicate that this agitation affected the size of the fat globules and the result was a reduced cream layer.

Preliminary trials upon separated milk, agitating for several hours first the cream and then the skimmilk and recombining to the original percentage indicated that the agitation apparently had but little effect when exerted on the cream, but when exerted on the skimmilk resulted in very noticeable decreases in the cream layer.

TABLE 5

Volume of cream rising on pasteurized milk made from viscolized cream and untreated skim milk

TREATMENT	TEMPERATURE	PER CENT OF TOTAL VOLUME APPEARING AS CREAM HOURLY AFTER SETTING											
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	8 hours	10 hours	12 hours	14 hours	16 hours	18 hours
Raw milk before separation, 4 per cent fat	50	10	11	11	11	11	11	12	12	12	13	13	14
	35	10	13	18	21	21	21	21	21	21	21	20	20
Pasteurized milk made from 45 per cent cream viscolized 2500 pounds pressure 4 per cent fat	50	10	25	26	26	26	25	25	24	24	23	22	22
	35	0	0	31	31	31	31	30	29	29	29	28	27

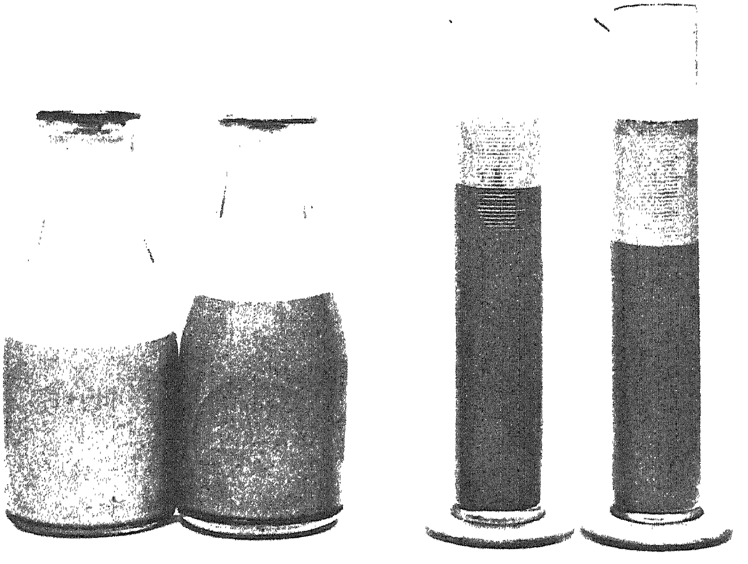
In the minds of the authors these results combined with those mentioned above indicate that it is not the fat of milk so much as it is the solids carried in the serum, which are affected by milk plant operations and which when acted upon mechanically bring about variations in the creaming ability of milk.

To corroborate these results a sample of milk was viscolized at 2000 pounds pressure and its creaming ability compared with a check sample. The volume of cream rising on the raw milk was 10 per cent, while that on the viscolized milk was 2 per cent.

Milk testing 4 per cent was then separated and the cream viscolized at 2500 pounds pressure before reconstructing. The results are indicated in Table 5.

DISCUSSION OF RESULTS

The results of eight trials showed that a slight decrease occurred when the milk was clarified at 55°F. In four trials in which the



No. 1 No. 2 No. 3 No. 4

PLATE 1

VOLUME OF CREAM RISING ON RAW MILK AND ON MILK MADE FROM VISCOLIZED CREAM AND UNVISCOLIZED SKIM MILK

Milk pasteurized at 142°F. for thirty minutes
No. 1. Milk made from viscolized cream, 4 per cent fat
No. 2. Raw milk, 4 per cent fat
No. 3. Raw milk, 4 per cent fat
No. 4. Milk made from viscolized cream, 4 per cent fat

milk was heated to 90°F. before clarification there was no decrease in creaming ability. In three out of four trials there was no decrease in the volume of cream when the milk was heated to 90°F. after clarification. It would seem logical to conclude that milk heated to pasteurization temperature after clarification would not have its creaming ability lessened due to the clarifier.

Pumping milk while cold did not lessen its creaming ability. The average of seven trials in which hot milk was pumped showed but a small loss.

Hot milk subjected to agitation showed a gradual loss in creaming ability. The loss was very small for the first two hours, but after that it amounted to 20 to 65 per cent.

To prevent loss in creaming ability of pasteurized milk, it should be cooled as quickly as possible with the least possible agitation. When milk is cooled in the pasteurizer considerable loss occurs, the amount depending on the type of pasteurizer, and amount of agitation to which the milk is subjected.

The fundamental cause of variation in creaming ability of milk will possibly be found in the milk serum, and not the butterfat, as has long been the opinion.

DAIRY NOTES

The Western Dairy Instructors Association has been authorized to form a Western Section of the American Dairy Science Association, with W. M. Regan of California as president.

Agricultural bacteriology in Italy, France and England as seen by a tourist.[1] R. S. Breed, New York Agricultural Experiment Station, Geneva, N. Y.

This paper is a brief review of the impressions gained by a recent visit to a series of European institutions engaged in research in agricultural and general bacteriology. In Italy, these included the three agricultural universities known as the Technical Higher Schools of Agriculture at Portici (near Naples), Perugia (Central Italy) and Milan Northern Italy). Visits were also made to the International Institute of Agriculture at Rome, the Experiment Station for Cheese Making at Lodi near Milan, the Animal Husbandry School at Reggio-Emilia and the Agricultural Experiment Station at Modena. As these are representative institutions out of a large number of institutions for agricultural education and research, as the work done is of high grade, is apparently in a position to secure reasonable support, and as a fine spirit of work seemed to pervade all of them, the impression gained was that the Italian group of workers would continue to contribute more than their full share to the sum of human knowledge gained by study in this field. The barrier of language between Italy and the U. S. A. is a serious one and has led Americans to undervalue the type of work that has been done in this progressive country.

A much longer stay in France showed that while very fine work was being done at a few institutions, there was no such extensive series of institutions doing high grade work in agricultural bacteriology as in Italy. Just as in the field of medical bacteriology, the Pasteur Institute takes the lead among French institutions for research in agricul-

[1] Read before the Central New York Branch of the Society of American Bacteriologists, Ithaca, N. Y., October 27, 1923.

tural bacteriology. At the Institute in Paris, the work of Professor Mazé in bacteriology and plant physiology is noteworthy, while the most interesting new development at the Institute is the establishment of a laboratory of soil bacteriology at Brie-Comte-Robert under the leadership of Professor A. Winogradsky. A visit to this newly-equipped laboratory, thirty miles to the east of Paris showed him to be very happy to be again at work after the changes brought about by the war and especially happy to be again working at the Institute to which Pasteur himself had invited him many years before. Especially noteworthy, also, is the work being done at the National Institute of Agronomy in Paris. Visits were also made to the National School of Agriculture at Grignon, and to the Veterinary Colleges at Lyon and at Alfort. In general, research work in agricultural bacteriology seemed to be suffering from a lack of adequate national support and there seemed little indication of the vigorous activity that might have been expected in a reconstruction period. French energies seem to be almost completely occupied in the reconstruction of the devastated regions and in related problems.

In England the chief centers of interest to agricultural bacteriologists are to be found at Rothamstead (soil work) and at the newly-organized National Institute for Dairy Research at Reading. The term newly-organized may be used, for although this institute was founded in 1910 under the directorship of Dr. Stenbouse Williams, the war so prevented its development that it is only now moving into its well-equipped and permanent quarters at Shinfield near Reading. A visit was also made to a newly organized secondary school of agriculture, the Cannington Court Farm Institute near Bridgwater in Somerset County, and to the Institute for Research in Agricultural Economics at Oxford. In this brief visit to England, the impression gained was one of vigorous activity and interest. This has firm foundation in the acute realization of the need for the development of English agriculture that has been brought home by the war. Better financial support seemed to be forthcoming, which, with an available supply of well trained workers, promised much for the future. A good indication of the lively interest in progress along dairy lines is to be found in the fact that England sent the largest delegation of any country to the World's Dairy Congress.

Advanced registry news reports. G. C. White, Connecticut Agricultural College, Chairman of the Breed Relations Committee.

Several states have been issuing a condensed monthly report of cows under test for some time. These reports go chiefly to the owners of herds under test, the local farm press, county agents and extension specialists, test supervisors, and to the offices of the various breed associations.

The breed associations officials are interested in these reports and some of them make some use of this information. For this reason, more than a year ago, Mr. Baker of the American Jersey Cattle Club expressed a desire to see these reports appear in uniform tables.

As we have been urging the breed associations to place their advanced registry systems upon a uniform basis, the Breed Relations Committee of the Official Testing Section of the American Dairy Science Association feel that Mr. Baker's wish is most reasonable. The fact that some states make exchanges of these reports is another good reason for having them uniform. Accordingly, the Committee recommended a form for this purpose at the 1922 meeting, but this form has not as yet become widely disseminated. In order to get this before the state superintendents, this present statement is prepared.

Since January, 1923, the Connecticut Agricultural College has been issuing monthly reports, following the plan outlined by the Committee. A brief outline of the September report is submitted herewith:

[*First page*]

The Connecticut Agricultural College A. R. News

Vol. 1, No. 9 Department of Dairy Husbandry

For month of September, 1923. Storrs, Conn., October 15

Brief summary of the Honor List, giving number of cows qualified, total number of cows tested, number of breeders on Honor List and total number of breeders testing.

Brief note concerning National Dairy Show winnings of Connecticut Jersey and Holstein state herds.

List of completed records coming up to certain minimum records.

New state records.

[*Second page*]

CLASS	BREED	COW'S NAME AND NO.	OWNER	MONTH OF LACTATION	PRODUCTION FOR 2 DAYS		
					Milk	Fat	Fat
					pounds	*per cent*	*pounds*
S3	G	Anesthesia Faith of Hillstead 114354	Riddle	3	91.2	5.56	5.073
M	G	Nina II of Greenway 57920	Greenway	4	104.7	4.63	4.850
M	J	Gamboge's Emma of H. F. S. 324610	Barnes	2	83.2	5.82	4.846
M	H	Blackberry Farm DeKol Buttergirl 522527	Moseley	1	127.8	3.76	4.800

This page is continued with a total of 27 cows on the list.

[*Third page*—continuation of page 2]

[*Fourth page*]

List completed. Also the list of the breeders on the Honor List giving name, address, total cows on test and total cows on the Honor List. This list identifies more completely the ownership of animals on the Honor List.

These lists are mimeographed and the pages are punched together. Placing the month, or the volume and number at the top or bottom of each page will be a convenience in helping to keep the reports together in case the pages become separated.

This "News" was started this year in Connecticut for the first time. The interest shown by the breeders and others, we feel justifies the time and expense of getting it out. It is mailed on the 15th of the month following the test period and is circulated before the information is received through any other channel. Furthermore, the breeder keeps informed concerning the progress in other breeds than his own.

The requirements for admission to the Honor List in Connecticut follow:

	lbs. fat in two days.
Mature cows (5 years or over)	4.000
Four year olds	3.635
Three year olds	3.270
Two year olds	2.905

The standard of requirements will naturally vary according to the number of cows on test in the various states.

Where it is preferred to publish the lists by breeds, the animals can be so classified, retaining this same form for presentation.

Some may wish to publish the total yields for the months. This can be done with the form as presented here, but the report will probably not be ready so early if complete monthly yields are used.

It should be understood by the breeders that any errors discovered by the cattle clubs or others are subject to correction.

REVIEW OF DAIRY LITERATURE

A Review of the Fourth Edition of the Standard Methods of Milk Analysis. E. G. Hastings.

A number of scientific associations in this country have been active in the development of methods of analyses, both chemical and bacteriological, to be used in controlling the quality of many products. The methods may relate to the commercial value of the products or to their healthfulness. Probably foremost among these associations in the development of methods for controlling healthfulness of foods, especially water and milk, has been the American Public Health Association through its Laboratory Section.

The Association published the first edition of "Standard Methods for the Bacteriological Examination of Milk" in 1910. The second edition was issued in 1916, and the third in 1920. The fourth which is entitled "Standard Methods of Milk Analysis" since it contains both bacteriological and chemical methods was published in 1923. It includes the bacteriological methods formulated by committees of the Laboratory Section of the American Public Health Association, of the American Dairy Science Association, and of the International Association of Dairy and Milk Inspectors. Doctor R. S. Breed of the New York Experiment Station, Geneva, has acted as referee for these various committees. A large part of the credit for the development and compilation of the methods should be given to him.

The present edition consists of a paper bound pamphlet of 40 pages. The first 24 pages are devoted to the bacteriological methods. These include the macroscopic colony count (the Petri plate method) and the microscopic count of bacteria (the Breed method) both of which are official methods.

The discussion of the first method includes the composition of the medium employed, its reaction and method for the adjustment thereof. Two procedures for the preparation of preparing agar are given. The procedure to be followed in plating, incubation, and counting the plates is presented. The common sources of errors in making plate culture counts are discussed as is the manner of reporting results.

The microscopic count of bacteria is presented under the heads of apparatus required, preparation of films of dried milk, standardization of the microscope, counting, sources of errors, and reporting results.

The description also includes two provisional methods, the microscopic colony count developed by Doctor W. D. Frost of the University of Wisconsin, and the reductase test or the methylene blue reduction test as it is more commonly called.

It should be recognized that each of these methods will enable the analyst to divide the milks examined into a number of grades which are differentiated by the number of bacteria in the milk. In a general way all give the same information in regard to the milks to which they are applied. On the other hand, one cannot expect that the number of bacteria found in a particular sample of milk through the use of one method will be the same as when another method is used. The number will usually be of the same order of magnitude, but now and then wide differences must be expected. This is due to the fact that the bacterial flora of milk consists of many kinds, some of which will be detected by one method and not by others. The variation is likely to become pronounced when the milk is heavily contaminated from special sources which are not constantly present on all farms. Examples of such sources are inflamed udders and milking machines, the latter giving a somewhat different type of contamination than utensils in general, when the parts are handled in certain ways.

There is no doubt but that each of the methods presented is of great value. One can best be used under a certain set of conditions, another under a different set of conditions. No one method is best. Each is good. Space does not permit a discussion of the conditions under which each of the various methods can best be used.

The sediment test is also described as is the detection of specific pathogens in milk.

The chemical methods are those adopted by the Association of Official Agricultural Chemists. They include the methods for the determination of casein, albumen, lactose, fat, added water, and for the detection of the common preservatives and coloring matters that may be added to milk.

The pamphlet can be obtained from the American Public Health Association, 370 Seventh Avenue, New York City. The cost is 40 cents per copy postpaid. Everyone interested in the control of milk should have a copy as should all engaged in the examination of milk for any purpose whatever. Teachers should also make use of the methods in their class work rather than to present other methods not officially recognized in this country.

CONDENSED AND POWDERED BUTTERMILK FOR DAIRY CALVES

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Received for publication February 9, 1924

On farms selling whole milk calf raising is a serious problem. The market value of whole milk under these conditions is usually so high as to practically prohibit feeding it to the calf and feeding skimmilk is likewise impracticable because of the inconvenience of separating the milk and marketing both cream and milk. Farmers under these circumstances instead of raising calves usually depend upon purchasing cows with which to maintain their herds, with the result that herd improvement is at a standstill. That a relatively large number of farmers are represented in this class is indicated by census figures which show that approximately 54 per cent of all the calves born in this country are the offspring of cows the milk of which is sold as whole milk from the farm.

Commercial calf-meals and so-called milk substitutes have been used for calf feeding for a number of years. In general, however, they have been found too expensive and in most cases also they have proved unsatisfactory except when milk feeding has been continued during the first two to four months of the life of the calf.

Methods of raising calves on a limited amount of milk have also been demonstrated at several state experiment stations (1, 2) as has the use of powdered skimmilk and malted milk (3, 4). These methods, however, are relatively expensive and consequently their use in practice will be limited.

Rapid progress has been made in recent years in the development of mechanical means for concentrating buttermilk either into a condensed or powdered form. This has made possible

the utilization of an excellent food product which in the past has been largely wasted. From an insignificant beginning this industry has grown to mammoth proportions. The estimates of the Bureau of Markets (5) for the year 1921 give a total production in the United States of approximately 30,000,000 pounds of condensed or semi-solid, and about 8,000,000 pounds of dried buttermilk. Since then the output of these products has greatly increased, and still only a small percentage of the total amount of available raw product is so utilized.

Why not utilize this concentrated product for calf feeding? The fact that raw buttermilk is practically a waste product in the sections where produced makes its ultimate cost in condensed or powdered form comparatively low. Also the use of ordinary raw buttermilk as a calf feed is not an uncommon practice in certain dairy sections of the world. Hill (6) in his history of the Guernsey breed states that calves on the Isle of Guernsey are fed on the milk from the churn from the time they are but a few days old until able to subsist on coarser feeds. In Ireland, Hanly (7) states that calves are raised successfully on buttermilk. Neither is the practice unknown in this country, as calves on farms located near creameries are sometimes raised on the milk from the churn. The experimental work of Otis (8) also indicated that this practice is successful. In his experiments one lot of 10 calves fed on buttermilk made an average daily gain of 1.79 pounds as compared with 2.02 pounds by a group on skimmilk. He found also that the buttermilk lot had less digestive trouble than the skimmilk lot.

Ordinary raw buttermilk has almost the same percentage composition and feed value as skimmilk. The chief difference is in the higher percentage of acidity which it usually contains. In the process of concentrating it, certain chemical and physical changes take place. These, however, are not of such a nature as to seriously, if at all, decrease the digestibility and food value of the product. It would therefore seem that it should be entirely satisfactory as a calf feed.

EXPERIMENTAL

The object of the experiment reported here was to determine whether calves would make satisfactory growth when fed concentrated buttermilk products in place of skimmilk. The general plan was to rear the calves on condensed or dried buttermilk, properly diluted and fed in the same manner as skimmilk supplemented by a good hay and grain ration. Ten calves in all were used.

In the first experiment two groups were used. The first group consisting of a purebred Jersey male and a grade Guernsey heifer

TABLE 1

Feed consumed to six months of age. Calves raised on semi-solid and powdered buttermilk

NUMBER OF ANIMAL	BREED	AGE AT WEANING	WHOLE MILK FROM FIVE DAYS OF AGE	POWDERED BUTTER-MILK	SEMI-SOLID BUTTER-MILK	GRAIN	ALFALFA HAY
		days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
E-7	Pbd. J.	106	250		186	376	262
E-8	G. Gr.	105	250		186	399	278
E-9	G. Gr.	150	268	135.1		363	237
E-10	G. Gr.	150	243	138.7		391	242
E-11	Pbd. J.	150	156	147.7		430	255
E-12	H. Gr.	70	201	65.0		497	500
E-14	H. Gr.	150	176	156.2		406	450
E-15	H. Gr.	70	180	51.7		452	433
E-16	H. Gr.	150	140	154.3		466	414
E-17	H. Gr.	70	146	60.8		464	475

calf was fed the semi-solid or condensed form of the buttermilk to 105 days of age. The second group received powdered buttermilk. In this lot were 2 grade Guernsey heifers and a purebred Jersey bull calf. They were weaned at 150 days of age.

The second experiment was begun after the first had been completed. The object was to secure data from a larger number of animals and to compare the results obtained from early weaning with that of late weaning. Five grade Holstein calves received powdered buttermilk. Three of them were taken off the buttermilk at 70 days of age while the other 2 continued to receive it until they were the age of 150 days.

The semi-solid buttermilk used was purchased from the Fairmont Creamery Company, Sioux City, Iowa. As will be shown by table 2, this product is approximately three times as concentrated as the original buttermilk. It was fed in a diluted form being mixed in the ratio of 1 part buttermilk to 3 parts by weight of warm water. This gave a mixture with practically the same composition as ordinary skimmilk.

Two lots of powdered buttermilk were used. That fed to the Guernsey-Jersey group was obtained from the Fairmont Creamery Company, Sioux City, Iowa. The Holstein group used in the second experiment was fed a product manufactured by the Collis Products Company, St. Paul, Minnesota. The

TABLE 2

Comparison of the semi-solid and powdered buttermilk used

	FRESH BUTTERMILK*	SEMI-SOLID BUTTERMILK	POWDERED BUTTERMILK
	pounds	*pounds*	*pounds*
Total solids	9.4	30.51	92.11
Water	90.6	69.49	7.09
Protein	3.6	11.14	35.00
Fat	0.1	0.84	2.86
Nitrogen free extract	5.0	15.80	43.59
Ash	0.7	2.73	11.46
Calcium		0.449	2.73

* Feeds and Feeding, 18th ed. p. 713.

composition of the products used together with the average analysis of buttermilk for comparison is given in table 2. This table indicates that these products contained about 9 times as high a percentage of solids as common buttermilk. Consequently to give them approximately the same composition as skimmilk 1 part of the powder was dissolved in 9 parts by weight of warm water.

Both the semi-solid and the powdered lots were typical of the product made from buttermilk of the quality common in creameries of the so-called centralizer type where the cream is transported a considerable distance and is ordinarily neutralized before churning. That a neutralizer was used is indicated by the

relatively high calcium content. When computed on the basis of the percentage present in the dry matter the concentrated products contain approximately twice the amount of calcium found in ordinary skimmilk. The addition of lime, however, is not objectional from a nutrition standpoint and may increase rather than decrease the feeding value of these products. However, in case a magnesium compound was used as a neutralizer in the cream the added mineral matter might be detrimental on account of the magnesium calcium antagonism which has been recognized in recent years. It has been shown that an excess of magnesium in the food intake results in the elimination of calcium.

As in the case when calves are to be raised on skimmilk, whole milk feeding was continued during the first ten days to two weeks of their lives or until they appeared sufficiently vigorous to stand a change in their diet. The change from whole milk to buttermilk was always made gradually. The method of feeding both the semi-solid and powdered forms of the buttermilk after the addition of the water was identical to that of skimmilk feeding.

In addition to the buttermilk the calves were given all the alfalfa hay they would eat besides receiving a grain ration consisting of 4 parts by weight of cornmeal, 1 of wheat bran, and 1 of linseed oilmeal. The amount of grain was usually limited not to exceed 5 pounds per day per calf at any time up to six months of age.

The animals were kept in separate pens at night and turned out for exercise in a dry lot during the day. Their growth in weight and height was determined by weights taken at ten day intervals and height measurements every 30 days. The growth of the animals was compared to the normal growth curves for females of the dairy breeds as published by one of the authors (9). These normal growth curves are used in place of check animals in investigations concerning growing dairy animals at this experiment station. Unfortunately no normal growth curves are yet available for the Guernsey breed or for males of any breed. For this reason the comparisons in figure 1 are with the Jersey normal. Since one group consisted of 2 Guernsey females and 1 Jersey male, and the other group of 1 Guernsey female and 1 Jersey

male, the results as shown in the figure give the appearance of being somewhat better than they really were. The results as expressed in figures are given in table 3.

Table 1 gives the age of weaning and the feed consumed up to the age of six months. Figure 1 and 2 show the growth curves for the various groups in their relation to the normal curve. The figures indicate that all the calves made excellent growth

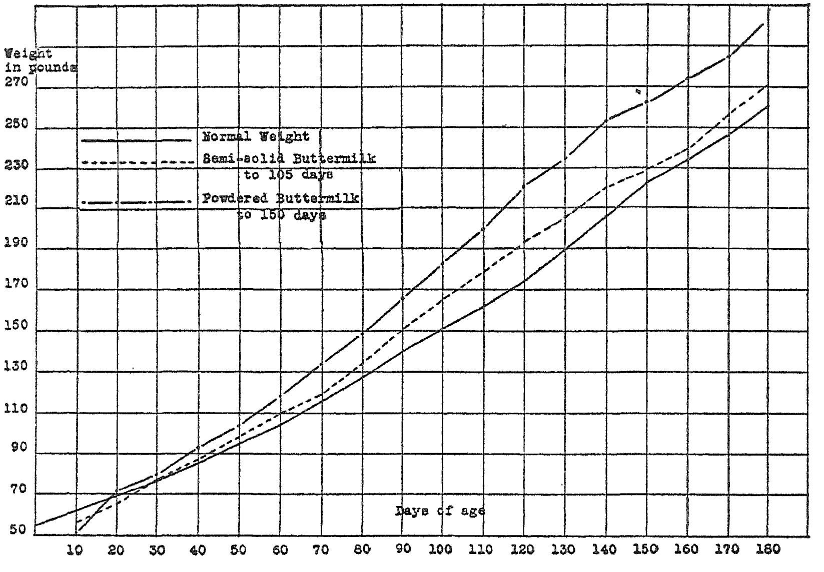

FIG. 1. GROWTH OF CALVES RAISED ON SEMI-SOLID AND POWDERED BUTTERMILK RATIONS COMPARED WITH NORMAL GROWTH

The broken lines represent the average weight by groups. Both groups were above normal during the entire period. The growth of the lot receiving semi-solid buttermilk was below that of the powdered buttermilk lot, partly as the result of earlier weaning.

while on the buttermilk ration. This is especially true of the Jersey-Guernsey group weaned at 150 days of age. That calves weaned at 70 days of age receive a set-back from which they do not entirely recover by the time they are six months old is shown in figure 2. There is, however, not enough difference in weight at six months of age between them and those weaned at 150 days to justify the longer period of buttermilk feeding. Table 3 gives

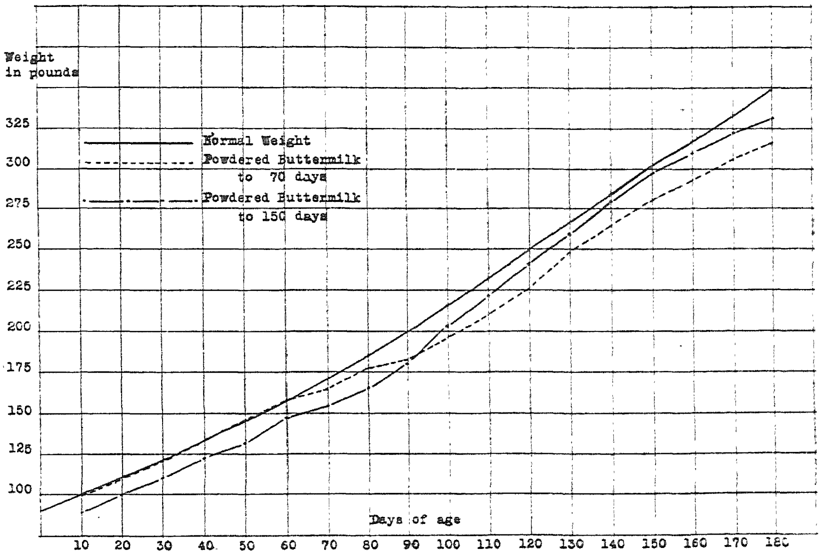

FIG. 2. THE GROWTH OF CALVES RAISED ON POWDERED BUTTERMILK COMPARED WITH NORMAL GROWTH

The broken lines represent the average weight by groups. One group was weaned at the age of 70, the other at 150 days. The earlier weaning resulted in a temporary decrease in the rate of growth. Both groups were slightly below normal at 180 days of age.

TABLE 3

Record of weights. Weights at beginning and close of experiment compared to the normal

NUMBER OF ANIMAL	BREED	WEIGHT AT TEN DAYS OF AGE	WEIGHT AT SIX MONTHS AGE	DAILY GAIN TO SIX MONTHS	NORMAL DAILY GAIN TO SIX MONTHS
		pounds	*pounds*	*pounds*	*pounds*
E-7	Pbd. J.	56	267	1.24	1.16
E-8	Gr. G.	58	275	1.28	1.16
E-9	Gr. G.	64	283	1.29	1.16
E-10	Gr. G.	72	297	1 32	1.16
E-11	Pbd. J.	51	332	1.65	1.16
E-12	Gr. H.	110	327	1.27	1.46
E-14	Gr. H.	80	317	1.39	1.46
E-15	Gr. H.	96	299	1.19	1.46
E-16	Gr. H.	95	344	1.46	1.46
E-17	Gr. H.	92	320	1.34	1.46

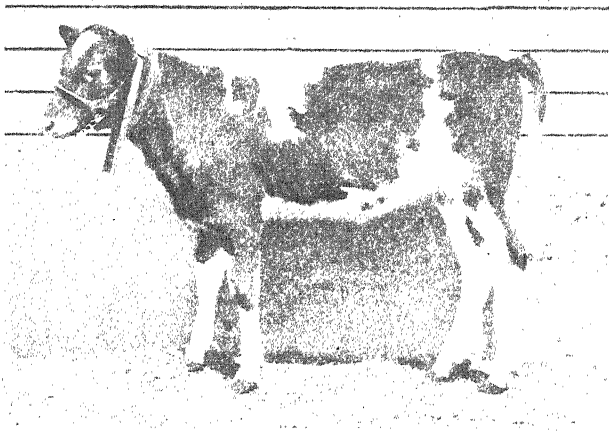

FIG. 3. GRADE GUERNSEY HEIFER. AGE FIVE MONTHS. RECEIVED POWDERED BUTTERMILK UNTIL WEANED AT 150 DAYS OF AGE

Weight at six months 283 pounds. Daily gain to six months 1.31 pounds. Feed consumed up to six months, whole milk 268 pounds, powdered buttermilk 135 pounds, grain 263 pounds, alfalfa hay 237 pounds.

FIG. 4. GRADE HOLSTEIN HEIFER. AGE SIX MONTHS. RECEIVED POWDERED BUTTERMILK TO 70 DAYS OF AGE

Weight at six months 327 pounds, daily gain up to six months 1.27 pounds, normal daily gain to this age 1.46 pounds. Feed consumed to six months, whole milk 201 pounds, powdered buttermilk 65 pounds, grain 497 pounds, alfalfa hay 500 pounds.

the weights of the individual animals at the close of the experiment and the gain per day compared to the normal.

All the calves used in this experiment were unusually free from sickness or digestion troubles. Not one of the calves were ever off feed after getting started on the experiment. No trouble was experienced in getting them to take the buttermilk as prepared. Rather they seemed to prefer it to whole milk. Scours or indigestion was never present. A slightly greater degree of looseness of the bowels was apparent among all the calves during the period of buttermilk feeding than is common when skimmilk is fed. This, however, did not affect their vigor or vitality but rather increased it. The calves were as a whole sleek-coated and thrifty, appearing the equal in size and condition to calves raised on typical farms.

ACKNOWLEDGMENT

A more complete report of the details of the first experiment are found in a thesis for the degree of Master of Science, University of Minnesota, by R. H. Lush who supervised the details of this portion of the investigation.

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A STUDY OF THE BIRTH WEIGHT AND GESTATION OF DAIRY ANIMALS

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The birth weight of a calf is always of interest to breeders. In spite of this fact there is very little definite information available concerning the factors that influence the birth weight of calves. This paper is the result of a study of the calves dropped in the Kansas State Agricultural College dairy herd. This herd is made up of representatives of the four major dairy breeds.

REVIEW OF PREVIOUS WORK

Eckles (1) and McCandlish (2) have recently published articles on this subject in which they have reviewed previous work. Eckles (1) reported upon 433 calf weights obtained from the dairy herd at the University of Missouri over a period of twelve years. He states that breed is the most important factor in influencing the weight of calves at birth and that the male calves average from 5 to 8 per cent heavier than females. His data indicate also that calves from immature cows are smaller than those from mature cows and that cows in advanced age produce smaller calves than cows in the prime of life. His experience prompted the conclusion that the sire had but little influence upon the size of the calf except in the case of cross bred animals when the influence of the sire becomes evident. He states also that the nutrition of the cow during gestation does not influence the size of the calf except under most extreme cases. Eckles found no definite correlation between the gestation period and the size of the calf except in the cases of extremely large or small calves. He presents data for Jersey calves which indicate that the heavier calves are carried longer than the lighter calves.

McCandlish (2) summarized 369 calf weights covering a period of fourteen years in the dairy herd at the Iowa State College. In this herd the male calves averaged 72 pounds which was 10 per cent heavier than the females. The females averaged 65 pounds and all calves 69 pounds. The Holsteins were largest, followed in order by the Ayrshires, Guernseys, and Jerseys. The scrub calves had the lowest average birth weight and the purebreds the highest. The grades used were very nearly the same as the purebreds in weight. McCandlish states that there was an increase in the birth weight of the calves from dams up to five years of age after which age there was an irregular decrease. He also found an increase in birth weight with an increase in the weight of the cows but this was not in direct proportion to the weight of the dam. He found a wide variation in the birth weight of calves dropped between April and October inclusive and the remainder of the year and infers that the calves dropped between April and October may be lighter than others. He further concludes that the gestation period has but little influence on the birth weight of calves.

EXPERIMENTAL WORK

This paper is a summary of data concerning calves dropped in the dairy herd at the Kansas State Agricultural College from September 1, 1910, to January 1, 1922. It has been the practice since September 1, 1910, to weigh all calves within six hours after they were dropped and to weigh the dam of the calf at the same time. The dairy herd is made up of purebred animals from the four major dairy breeds, some grade Holsteins used in the dairy herd and an equal number of grade Holsteins raised under experimental conditions. In the summary given they have been classified as mentioned above.

All calves from gestation periods of less than 260 and more than 295 days duration have been eliminated. The weights of twin calves are not used in the summaries. The weights on all calves have been eliminated that did not have the weight of dam recorded.

DISCUSSION OF BIRTH WEIGHTS

Table 1 is a summary of the data obtained on 521 calves and their dams in the dairy herd at the Kansas State Agricultural College. It will be seen that the average weight of Jersey calves is 57 ± 0.9 pounds, of Guernsey calves 64 ± 0.9 pounds, Ayrshire calves 72 ± 1.03 pounds and of Holstein calves 95 ± 1.57 pounds.

The male calves averaged from 4 to 11 pounds heavier than the females, the average difference for the four breeds being 7.8 per cent. In the case of the Guernsey, Holstein and experimental Holsteins we find the difference between the weights of the bull calves and the heifer calves to be from 3.8 to 4.2 times the probable error which indicates a marked difference between the weights of bulls and heifers in the group studied and would mean the same to be true for other groups under similar conditions.

The relation of the weight of the calf to the weight of the dam followed quite closely the average weight of the breed, but did not increase in proportion to the size of the dams. In the case of the experimental Holsteins we find a marked increase in the relation of the weight of the calf to the dam. This may be due to the method of feeding the animals as will be shown later.

Table 2 is presented for a comparison of the birth weights of the calves at the Missouri, Iowa and Kansas herds. While the results are quite uniform it is important to note that the weights from the Kansas herd are consistently higher than from the other two herds. In most cases, however, the difference in the weights from the Kansas herd is no greater than the difference between the birth weights in the other two herds but they are, with one exception, always higher than the others. It is hard to explain this difference in weight. The weights of the dams of the calves at the Missouri and Iowa stations are not available but in comparing the weights of the Kansas cows with the weights reported by Eckles from all available sources the Kansas cows are larger except for the Guernsey breed. The size of the animals may in a way account for the difference in weights of the calves. The Guernsey herd has been made up of a large proportion of young animals. These heifers, mostly from one sire, have not reached the size desired for the breed.

TABLE 1

Birth weights of calves from Kansas State Agricultural College herd

BREED	MALES		FEMALES		BOTH SEXES		AVERAGE WEIGHT OF DAMS	PER CENT OF WEIGHT OF DAMS
	Number	Weight	Number	Weight	Number	Weight		
		pounds		*pounds*		*pounds*	*pounds*	
Jersey	53	59±1.17	39	54±1.3	92	57±0.90	908±10.2	6.3
Guernsey	50	67±1.18	54	60±1.17	104	64±0.90	948±12.2	6.7
Ayrshire	54	75±1.35	61	69±1.44	115	72±1.03	1033±14.5	6.9
Holstein	34	101±2.12	41	90±1.96	75	95±1.57	1314±22.1	7.1
Grade Holstein	31	91±1.8	38	87±2.09	69	89±1.37	1115±17.1	7.7
Exp. Holstein	31	96±2.15	35	85±1.52	66	90±1.43	1042±18.0	8.6
Weighted average of totals	253	78.5	268	72.8	521	75.5		

TABLE 2

Comparison of weights from three college herds

BREED	MALES			FEMALES			BOTH SEXES			PER CENT OF WEIGHT OF DAMS		
	Missouri	Kansas	Iowa	Missouri	Kansas	Iowa	Missouri	Kansas	Iowa	Missouri	Kansas	Iowa
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Jersey	58	59	55	53	54	52	55	57	54	6.5	6.3	6.1
Holstein	93	101	97	88	90	89	90	95	94	8.0	7.1	7.7
Ayrshire	73	75	68	65	69	65	69	72	66	6.9	6.9	7.8
Guernsey		67	66		60	61		64	64		6.7	7.1

TABLE 3

All available weights of dairy calves

BREED	NUMBER OF CALVES	AVERAGE WEIGHT	DAMS	PER CENT OF WEIGHT OF DAMS
		pounds		
Jersey	418	55	877	6.2
Holstein	513	90	1146	7.8
Ayrshire	230	71	1012	7.0
Guernsey	238	65	965	6.7
Brown Swiss	5	100	1123	8.9
Shorthorn	30	73	1216	6.0
Total all breeds	1434			

Table 3 is a summary of all the available weights of calves for the dairy breeds. The data from the Iowa and Kansas herds is added to that reported by Eckles. The average weights of the calves and the weights of the dams were obtained by finding the weighted average from the data from the different sources. This summary compares quite favorably with the original summary reported by Eckles except in one case. The average weight of Guernsey calves has been reduced from 71 to 65 pounds, and the average weight of the dams from 996 to 965 pounds due to the addition of 104 Guernsey weights from the Kansas herd and 77 from the Iowa herd.

Henry and Morrison (3) report a summary of the weights of calves from the Connecticut, Missouri and Wisconsin stations. Their table includes the results of the Missouri station as does table 3. The results are essentially the same as table 3 with the exception of the weight of Guernsey calves which is 6 pounds heavier than given in table 3.

Eckles has shown that the size and thrift of the calf is, within limits, independent of the feeding of the dam. This has also been observed in practice since it is not uncommon for cows in high condition of flesh to produce small calves and on the other hand thin cows may produce large vigorous calves. These observations are verified by the data presented in table 4 giving the weights of calves and dams of 39 animals used in a feeding experiment from birth through two lactation periods. The animals in one lot were fed alfalfa hay and in another lot alfalfa hay and silage after being taken from milk. The last two lots were fed alfalfa hay, silage and grain, one lot calving at thirty months and the other at twenty-four months of age. The animals in the last two lots received a liberal grain ration. The animals fed alfalfa hay weighed less than the other lots. Their calves were somewhat smaller in weight but were larger in proportion to their dams. Eckles has likewise reported light fed Jerseys and light fed Holsteins whose calves show a much greater proportion to the weights of the dams than animals of the same breeds on full feed.

Maid Henry Pontiac, a purebred Holstein in the Kansas herd, while being fitted for an Advanced Registry test weighed, on six days before freshening, 2150 pounds, this being the limit of weight on the barn scales. After freshening she weighed 1870 pounds and her bull calf weighed 119 pounds which is 6.3 per cent of the weight of the dam. Another case of a cow highly fitted that proved disasterous to both the cow and calf was that of Blossom Mechthilde, a purebred Holstein weighing 1600 pounds bred to a Holstein Bull weighing 2100 pounds that produced a bull calf which weighed 170 pounds within an hour after birth. This is the heaviest calf recorded in this herd. Twin Holstein calves have been recorded in our herd, the total weight of which was 174 pounds and in another case 163 pounds but

TABLE 4

Influence of nutrition of dam upon weight of calves

FEED	NUMBER OF ANIMALS	WEIGHT OF DAM	WEIGHT OF CALF	PER CENT
		pounds	*pounds*	
Alfalfa hay	12	917	84.8	9.24
Alfalfa-silage	8	966	88.1	9.11
Alfalfa-silage-grain 30	9	1076	90.5	8.4
Alfalfa-silage-grain 24	10	1032	93.2	9.03

the above calf is the heaviest to date. A bull calf weighing 132 pounds was dropped by a cow weighing 1100 pounds, while the cow was on a so-called light fed ration receiving alfalfa hay and silage but no grain and had been on this feed since being weaned. Long time feeding on restricted diets apparently influences the size of a cow's offspring, but feeding influences the size of a calf less than generally believed among breeders.

INFLUENCE OF THE SIRE

The sire is believed by some to influence the birth weight of calves and we frequently hear of a sire that is getting larger or smaller calves than others. In the case of a Holstein sire used in the Kansas herd we have noticed that without regard to the size of the dam the calves sired by Campus Sir Korndyke Quad

have been larger than others.[1] This is shown in table 5. Another difference is noticed in the case of Ayrshire bulls. Cavaliers College Master was a rather large rugged bull and his calves have averaged heavier than the calves from Melrose Good Gift.[1]

Table 5 does not give information that would warrant a very definite conclusion. From the actual weights reported and the observation of the authors it would seem that the ability to sire large calves is an individual characteristic. A Guernsey bull now in use in the Kansas herd is siring calves which to date average heavier than either of the two Guernsey bulls mentioned in the table.

INFLUENCE OF AGE OF DAM ON WEIGHT OF CALF

The age of the dam apparently has an influence upon the size of the calf. The calves from all the cows that have had more than 5 calves were averaged in the order of their birth as shown in table 6. The cows within the different breeds show an increase up to the fifth calf. This increase is not so apparent, however, when all the breeds are averaged. While the data are rather meager they seem to indicate that the first calves are smaller than the calves from the same cows when mature. The size of the calves from individual cows seem to decrease with the age of the cows after the sixth calf. This, however, is not so apparent in the table.

PERIOD OF GESTATION

The influence of the period of gestation upon the birth weight of calves is not marked. In table 7 is presented the data covering the length of gestation for the calves in the college dairy herd. The average weight of the calves and their dams is also given. It will be noted that there is a slight difference in the length of gestation due to breed. The Jerseys have an average gestation period of 284.3 days, the Guernseys 283 days, the Ayrshires 284.6 days and all Holsteins 281.0 days. When a direct average

[1] Not statistically significant.

TABLE 5

Influence of sire on weight of calves

BULL	BREED	WEIGHT OF BULL	NUMBER OF CALVES	AVERAGE WEIGHT OF CALVES
Melrose Good Gift	Ayrshire	1493	49	71 ±1.46
Cavaliers College Master	Ayrshire	1700	21	77 ±2.8
Bells Melrose	Ayrshire	1850	31	72.9±1.3
B. S. Stars and Stripes	Guernsey	1407	8	66 ±4.38
Langwater Benefactor	Guernsey	1400	53	66 ±1.1
Winnies Pogis Torono	Jersey	1289	37	61 ±1.3
Sult. Jolly King	Jersey	1362	35	56 ±1.4
Sir Carlotta P. C.	Holstein	2100	14	95 ±3.06
C. P. F. H. 6th	Holstein	2160	40	95 ±2.2
Campus S. K. Quad	Holstein	2090	23	100 ±3.7

TABLE 6

Relation of age of dam to weight of calf

CALVES	AYRSHIRE	GUERNSEY	JERSEY	HOLSTEIN
	pounds	*pounds*	*pounds*	*pounds*
1st	55	55	55	90
2nd	73	57	62	99
3rd	74	64	57	91
4th	80	61	56	105
5th	85	66	56	96
6th	77	68	58	102
7th	72	67	60	101
8th	64	70	59	93
9th		71	65	
10th		65		

TABLE 7

Length of gestation

BREED	BULL	NUMBER OF CALVES HEIFER	TOTAL	WEIGHT OF DAMS	BULLS	HEIFERS	AVERAGE GESTATION
Jersey	57	43	100	908	285.9±0.95	282.0±1.03	284.3±0.72
Guernsey	50	53	103	948	283.5±0.6	282.5±0.54	283.0±0.46
Ayrshire	52	61	113	1024	284.6±0.71	284.6±0.55	284.6±0.44
Holstein	37	41	78	1319	282.8±0.79	281.8±0.95	282.2±0.62
Grade Holsten	36	38	74	1156	281.2±0.86	280.8±0.89	281.0±0.68
Exp. Holstein	32	36	68	1042	280.6±0.76	280.2±1.12	280.4±0.69

is taken of all the animals the average period of gestation is found to be 282.4 days for all calves with 283.2 days for the bulls and 281.9 days for the heifers. Two gestation periods of 301 days each were recorded. The shortest gestation recorded was

TABLE 8

Relation of weight of calves to gestation period within breeds

CALVES	NUMBER	AVERAGE WEIGHT	AVERAGE GESTATION PERIOD
	Guernsey		
pounds			
40–49	6	45.8	276.8
50–59	22	55	282.3
60–69	45	64	282.3
70–79	23	73	285.1
80–89	6	81.5	285
	Jersey		
40–49	12	45.6	280.5
50–59	42	54.3	285
60–69	31	64.4	283.6
70–79	9	71	288.6
	Ayrshire		
50–59	8	54.6	279
60–69	31	63.9	284.2
70–79	53	74	284.2
80–89	13	83.4	287
90–100	8	93.6	289
	Holstein		
70–79	8	74	280.6
80–89	15	84	279
90–99	16	95	282
100–109	23	104.5	283.6
110–120	11	113	286

of 256 days duration. Fifteen pairs of twins from all four breeds had an average gestation of 273 days. The method of counting the days in the gestation was to add both the day the animal was served and the day the calf was dropped to the actual calendar days covered by the period.

The data in table 7 indicate that there is no very direct correlation between the size of the calf and the period of gestation when divided according to breeds. If there is a relationship it would seem that the smaller breeds carried their calves longer. This, however, is not borne out when the light calves are compared with the heavy calves within the different breeds. As shown in table 8 there seems to be a tendency for the heavier calves to be carried longer by their dams.

TABLE 9

Length of gestation periods for individual cows

	A. Canary Bell	A. Bangora	A. Bangora II	A. Johanna	H. 120	H. 114	J. Owls Design	J. College Tips	J. R. P. II	J. S. J. T.	G. Frances	G. Bernice Countess	G. M. M. Pink	G. B. C. II	J. Rosalpha
	days	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
	289	287	281	289	290	284	281	280	275	284	283	289	285	279	282
	288	281	284	289	281	285	285	283	256	291	277	282	288	288	292
	286	293	283	283	278	280	283	288	258	286	283	282	277	280	291
	276	290	279	285	277	282	288	285	290	301	274	291	286	278	293
	283	285	294	284	286	278	289	282	284	293	278	284	280	287	289
	281		287		280	288	286	292	289		284	284	282	280	
			289		283		287	288						282	
					279										
Average..	284	287	285	286	281	282	285	285	275	291	279	285	283	282	289

The difference in the length of the period of gestation for the different breeds, as shown in table 7 is contrary to results obtained by McCandlish (2) in a study of 360 gestations in the Iowa State College dairy herd. From this study he concludes that breed did not influence the period of gestation. He also states that the average length of gestation for the animals studied was 280 days. This is in accord with Wing (4) but is lower than the average of 1062 observations by Fleming (5) who reports an average gestation of 283 days.

Table 9 gives the length of gestation for 15 cows of the four dairy breeds in the college herd that have produced 5 calves

or more. No effort has been made to summarize the results according to the exact age of the cows but in comparing the different gestation periods it is apparent that there is a wide difference in the individual cows.

The two longest gestation periods observed were 301 days each. Several periods between 290 and 295 days were recorded but there may be some question in regard to the gestation of 301 days. It is not impossible that the 2 animals may have conceived to a later service that was not recorded. Gestations of more than 300 days have been reported previously but they are not common.

SUMMARY

1. The average weight of Jersey calves in the herd studied was 57 pounds, of Guernsey calves 64 pounds, of Ayrshire calves 72 pounds, and of Holstein calves 91 pounds.

2. The Jersey calves represented 6.3 per cent of the weight of their dams, the Guernseys 6.7 per cent, the Ayrshires 6.9 per cent and the Holsteins 7.8 per cent of the weight of their dams.

3. The male calves were 4 to 11 pounds heavier than the female calves. The average birth weight of all bull calves studied is 7.8 per cent heavier than the females.

4. Immature cows produced smaller calves than mature cows.

5. The birth weights recorded for dairy calves in the Kansas herd were consistently higher than the weights of calves reported from the Missouri and Iowa herds.

6. The nutrition of the dam had but little influence on the weight of the calf except in the case of cows on a restricted diet. In the case studied the dams on a restricted diet produced calves heavier in proportion than did well fed dams.

7. A sire may have the ability to influence the birth weight of a calf, but only to a limited degree.

8. The gestation period has no definite influence upon the size of the calf at birth. There are, however, within the different breeds, indications that the large calves are carried slightly longer than the smaller calves.

9. The average length of all the gestations studied was 282.4 days. The bulls were carried 283.2 days and the heifers 281.9. The breeds varied in the length of gestation period as follows:

	days
Jersey	284.3 ±0.72
Guernsey	283.0 ±0.46
Ayrshire	284.6 ±0.44
Holstein	281.0 ±0.15

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THE EFFECT OF HEAT ON THE ACTIVITY OF THE ENZYME PEROXIDASE AS FOUND IN MILK

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The enzyme peroxidase is the most widely distributed enzyme. It is found in all living organisms, both vegetable and animal tissue, and it has been said when a body fails to show the reaction of peroxidase it is dead. One peculiar property possessed by peroxidase is its resistance to decay, one of the most characteristic properties of living matter. It is still an open question whether peroxidase is a true enzyme, a question which cannot be definitely decided until more knowledge is at hand.

It is only by the action of the enzyme that furnish proof of their existence. Their actions can be investigated by studying the chemical nature of the bodies that are formed and also the rate of change and the conditions affecting it. The function of an enzyme is that of a catalyst, accelerating the action which has already begun, but never appearing among the final products. They merely increase the velocity when a chemical system strives to attain its final state. Under the name peroxidases are included those substances which are credited with the ability to accelerate oxidation processes. This action is going on within the organisms between organic peroxides and oxidizable compounds. The properties of peroxidase which concerns this article is the activation of hydrogen peroxide and the transferance of oxygen from the peroxide to other substances such as acid and other phenol derivatives.

The object of the following experiment was (1) to determine to what extent and at what temperature peroxidase as found in milk

[1] Student assistant, 1918.

is destroyed on heating; (2) to study the relation of the monomolecular law, or law of mass action to the rate of inactivation of the enzyme. The effect of heat on the peroxidase has been studied by J. J. Van Eck (Zeit. f. Untersuch der Nahrungsmittel, Band 22, 1911). We deemed it advisable to corroborate his conclusions and extend the application of the effect of heat on peroxidase. Use was made of the well known Storch reaction for heated milk which consists in adding para phenylenediamine

TABLE 1

Comparative scale

SCALE NUMBER	MILK		SCALE NUMBER	MILK	
	Raw	Sterilized		Raw	Sterilized
	cc.	*cc.*		*cc.*	*cc.*
1	0.10	9.90	17	3.50	6.50
2	0.20	9.80	18	3.75	6.25
3	0.30	9.70	19	4.00	6.00
4	0.40	9.60	20	4.25	5.75
5	0.50	9.50	21	4.50	5.50
6	0.75	9.25	22	4.75	5.25
7	1.00	9.00	23	5.00	5.00
8	1.25	8.75	24	5.25	4.75
9	1.50	8.50	25	5.50	4.50
10	1.75	8.25	26	5.75	4.25
11	2.00	8.00	27	6.00	4.00
12	2.25	7.75	28	6.25	3.75
13	2.50	7.50	29	6.50	3.50
14	2.75	7.25	30	6.75	3.25
15	3.00	7.00	31	7.00	3.00
16	3.25	6.75	32	7.50	2.50

and hydrogen peroxide to the milk. If peroxidase is present the enzyme will catalyse the oxidation of the paraphenylenediamine by the peroxide and the deep blue color of the oxidation product will appear. The peroxidase content depends on the degree and length of the heating period and the intensity of the blue depends in turn on the peroxidase content. Inasmuch as the shade of blue varies with the amount of peroxide and para-phenylenediamine the same quantity of these reagents was used in each test. The acidity also affects the rapidity of destruction of the enzyme, therefore, fresh milk was used in all our work.

In order to carry out the experimental work it was necessary to have a method by which the relative amount of peroxidase could be estimated. A comparative scale was made by diluting milk sterilized by heating to 95 to 98°C. for thirty minutes with raw milk in many different proportions.

The determination depending on the fact that the intensity of the blue color varies with the amount of peroxidase present. The same milk was used in the preparation of this comparative scale that was used later in the heating experiment. The dilution for making the comparative scale is shown in table 1.

For the purpose of testing the accuracy of this colorimetric determination, known dilutions were prepared and it was found that a variation of 0.25 to 0.5 cc. of raw milk could be detected. The errors were less in the lower concentrations of peroxides.

The plan of the heating experiment was to heat a sample of raw milk at a constant temperature taking samples at intervals and determining the peroxidase content by adding reagents and referring to the comparative scale. In this way data were secured for studying the rapidity of destruction of the enzyme.

APPARATUS, EXPERIMENTAL WORK AND RESULTS

The flask in which the milk was placed consisted of a wide mouthed bottle provided with a three-holed rubber stopper in which was placed a thistle tube for drawing off samples, a stirring rod for agitating the milk and a thermometer. In carrying out the experiment a constant temperature bath provided with a thermostat was used. The empty flask was placed in the bath until temperature was constant. Then the sample of milk was heated as rapidly as possible to a temperature 2° higher than that at which the experiment was to be conducted by pouring it into a thin flask immersed in boiling water or by carefully heating over a gas burner. The sample was then quickly transferred to the milk flask in the waterbath and about five minutes allowed for the temperature to become constant. Then the samples were drawn off at intervals and tests made with the results indicated in table 2. The hot samples coming from the milk flask was run into a test tube standing in ice cold water, thus stopping the destruction

almost immediately. The color tests were applied by adding 5 drops of a freshly prepared 2 per cent solution of para-phenylendiamine and 5 drops of a neutral 1 per cent solution of hydrogen peroxide to the milk samples, allowing them to stand thirty minutes before making comparison of colors. There is a gradual darkening of color after thirty minutes; so in order to offset this, the tests were applied to the heated samples and to the comparative scale at the same time. The temperature of the heated milk was taken immediately before the sample was withdrawn.

TABLE 2

Heating experiment

NUMBER	TEMPERATURE (NO. 1)	TIME	COLOR NUMBER	ENZYME CONCENTRATION
	°C.	*minutes*		
	70.3	0		
1	70.0	15	25	5.50
2	69.8	5	22–23	4.87
3	69.9	5	21–22	4.62
4	69.9	5	19	4.00
5	70.0	5	16	3.25
6	69.7	7	15	3.00
7	70.2	8	13–14	2.62
8	70.0	5	10–11	1.87
9	69.4	19	8	1.25

Taking the quantity of peroxidase in the raw milk as 10. The value 10 for the initial concentration is only relative and was chosen for convenience in the calculation. The volume of each sample of milk was 10 cc. It is seen from the results in table 2 that the per cent of peroxidase remaining active after heating the milk to 70°C. and holding at that temperature for five minutes was 55 per cent and the concentration decreased gradually on continuing the heating at approximately 70°C.

It was found that when the observed values of the quantity of peroxidase active at different intervals were plotted against time a curve was obtained similar to a logarithmic curve. It is well known from physical chemistry that the action of a true catalyst proceeds according to a definite law. This law is ful-

filled to a varying extent by enzymes, and this is the law of mass action. If a single type of molecules suffer on account of the action, the reaction may be stated by the following expression (1) $\log \frac{1}{t} \frac{E}{E-X} = K$, known as the monomolecular law, in which E represents the original concentration; X the quantity changed; t the time interval expressed in minutes from the beginning of the experiment; K is the velocity constant or reaction constant. Its value is retained no matter how far the reaction proceeds or what the original concentration may have been.

It may be desirable to construct a complete curve from the beginning of the destruction to the time of the last sample being taken. It is, therefore, necessary to know the time which would be required to bring the concentration of peroxidase down to that found at the time of first sampling and at the temperature at which the experiment was conducted. If it were possible to heat every particle instantaneously to the temperature of the experiment we could construct a continuous curve beginning with the initial concentration. This is impossible. If we make use of an average value of K it is possible to obtain a value for T or the time it would have taken to bring the concentration from the beginning to the time t if held at the temperature of the experiment. From the following mathematical expression derived from equation (1) by substituting the value of E, X, t and K we can arrive at a value for T (2). $T = \frac{\log 10 - \log (10 - X)}{K} - t.$

The value for T gives the time it would have required to reduce the peroxidase concentration from the initial concentration 10 to the concentration at the time when samples were taken at the temperature at which the milk was held. $T + t$ therefore gives the time required to reduce the peroxidase during the heating experiment after being heated in bath for a time t.

Table 3 gives the calculated value of the velocity constant K and the time T, also the calculated concentration from the average value of K and the average value of T.

The results of experiments conducted at different temperatures are embodied in tables 3, 4, 5, 6 and 7.

TABLE 3

Average temperature 69.9

TUBE NUMBER	TIME	ENZYME CONCENTRATION	K	T	CALCULATED CONCENTRATION
1	0	5.50		25.9	5.50
2	15	4.87	0.0243	26.1	4.91
3	5	4.62	0.0174	23.4	4.38
4	5	4.00	0.0212	24.7	3.90
5	5	3.25	0.0263	28.7	3.47
6	7	3.00	0.0225	25.2	2.96
7	8	2.62	0.0212	23.0	2.46
8	5	1.87	0.0269	32.5	2.19
9	19	1.25	0.0251	31.0	1.42
Average			0.023	26.0	

TABLE 4

Temperature 69.6°C.

TUBE NUMBER	TIME	ENZYME CONCENTRATION	K	T'	CALCULATED CONCENTRATION
1		2.70		66.8	2.71
2	5	2.45	0.0194	66.7	2.45
3	5	2.25	0.0182	66.2	2.23
4	5	2.00	0.0200	67.1	2.02
5	6	1.70	0.0220	69.5	1.79
6	5	1.55	0.0213	69.2	1.63
7	5	1.50	0.0189	65.8	1.48
8	5	1.40	0.0182	64.2	1.34
9	5	1.25	0.0188	64.9	1.26
Average			0.020	67.0	

TABLE 5

Temperature 72.4°C.

TUBE NUMBER	TIME	ENZYME CONCENTRATION	K	T'	CALCULATED CONCENTRATION
1		4.20		7.32	3.92
2	6	2.30	0.1004	7.40	2.17
3	5	1.00	0.1305	8.4	1.11
4	10	0.30	0.125	8.6	0.35
Average			0.12	8.0	

The curves represented in figure 1 show the rate of destruction of peroxidase on heating at a constant temperature and are plotted from the calculated values of enzyme concentration as

TABLE 6

Temperature 71.0°C.

TUBE NUMBER	TIME	ENZYME CONCENTRATION	K	T'	CALCULATED CONCENTRATION
1		7.00		7.8	6.93
2	10	4.75	0.0388	6.2	4.37
3	6	3.75	0.0390	5.3	3.32
4	4	2.50	0.0515	10.2	2.75
5	5	2.12	0.0478	8.7	2.19
6	5	1.87	0.0437	6.4	1.74
7	5	1.62	0.0418	3.6	1.44
8	5	1.12	0.0458	7.7	1.15
9	5	0.70	0.0510	12.8	0.87
10	5	0.62	0.0485	10.5	0.70
11	5	0.55	0.0463	8.0	0.55
12	5	0.42	0.0469	9.0	0.44
13	5	0.35	0.0461	7.8	0.35
Average............................			0.046	8.0	

TABLE 7

Temperature 70.8°C.

TUBE NUMBER	TIME	ENZYME CONCENTRATION	K	T	CALCULATED CONCENTRATION
3		5.50		14.9	5.07
4	5	4.75	0.0293	13.6	4.15
5	5	4.25	0.0258	11.4	3.40
6	5	3.25	0.0351	13.1	2.78
7	5	2.25	0.0447	17.3	2.28
8	5	1.75	0.0458	18.6	1.86
9	5	1.25	0.0493	22.0	1.5
10	5	0.75	0.0570	29.8	1.25
Average............................			0.04	17.0	

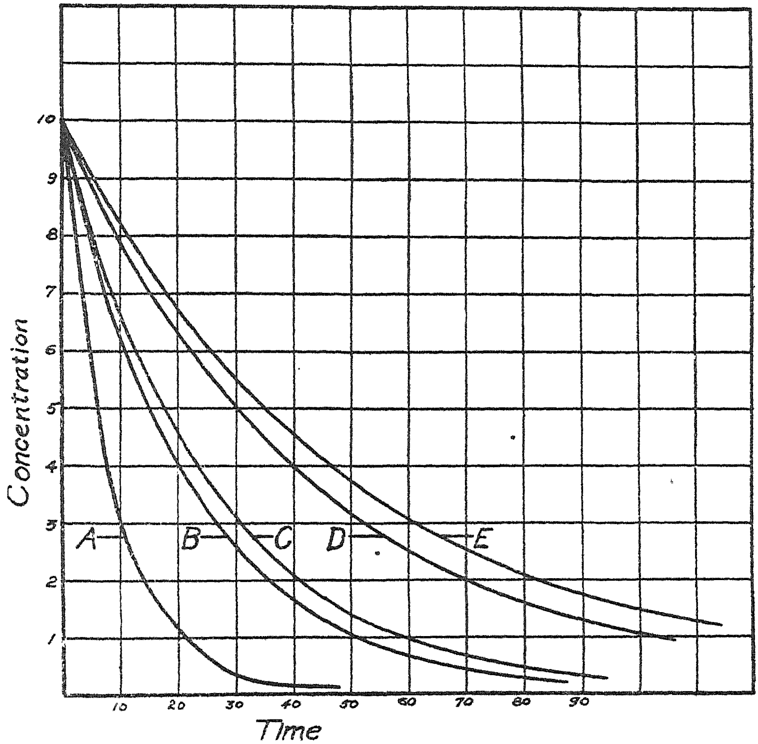
reported in the tables, 3, 4, 5, 6 and 7. It will be noticed that the experimental error in these observations in practically all cases is well within 0.2 to 0.3 cc. of active enzyme solution.

In addition to the foregoing tables the results of experiments at various temperatures is of interest when comparing the values of K and the destruction of peroxidase obtained for the different temperatures of heating the milk.

Fig I

Rate of Destruction of Peroxidase on Heating

A Temperature 72.4° C Temperature 70.8° E Temperature 69.6°
B " 71.0° D " 69.9°



The results as shown in table 8 indicate some practical application of these experiments. They express mathematically the relation between (1) period of heating, (2) degree of heating, (3) diminishing the activity.

The conclusion drawn from the data in the tables is valuable in studying the effect of pasteurization at different temperatures. It was found that when milk was heated to 62.5°C and held for twenty minutes produced no apparent effect on the peroxidase. In order to test the effect of flash pasteurization, a laboratory apparatus (fig. 2) was contrived by which the milk was heated to the required temperature and cooled in less than three seconds. The results shown in table 9 were obtained.

TABLE 8

EXPERIMENT NUMBER	TEMPERATURE	K	TIME REQUIRED TO DESTROY HALF OF ENZYME
	°C.		*minutes*
1	69.6	0.020	35
2	69.9	0.023	30
3	70.8	0.04	17
4	71.0	0.046	15
5	72.4	0.120	6

TABLE 9

HIGHEST TEMPERATURE	PEROXIDASE DESTROYED
°C.	*per cent*
85	92.5
85	92.5
80	42.5
76	40.0
64	Very slight

FIG. 2. APPARATUS USED IN HEATING MILK RAPIDLY TO THE TEMPERATURE AS SHOWN IN TABLE 9

Less than three seconds were required to heat the milk and again cooling to 30°C.

Explanation of parts of apparatus: 1 shows a 2000-cc. beaker holding the milk to be heated. The milk is syphoned into the condensor *2*, the flow regulated by a clamp. Steam under 10-pound pressure was passed through the condensor. The heated milk flowing over the thermometer *5* then passing through a short condensor *3*. The cooling was effected by passing under cooled salt-brine through this condensor. A 2-gallon candy bucket *4* contained the under-cooled brine. The heating of the milk was controlled by the rate of flow of milk through *2* and also by regulating the flow of steam.

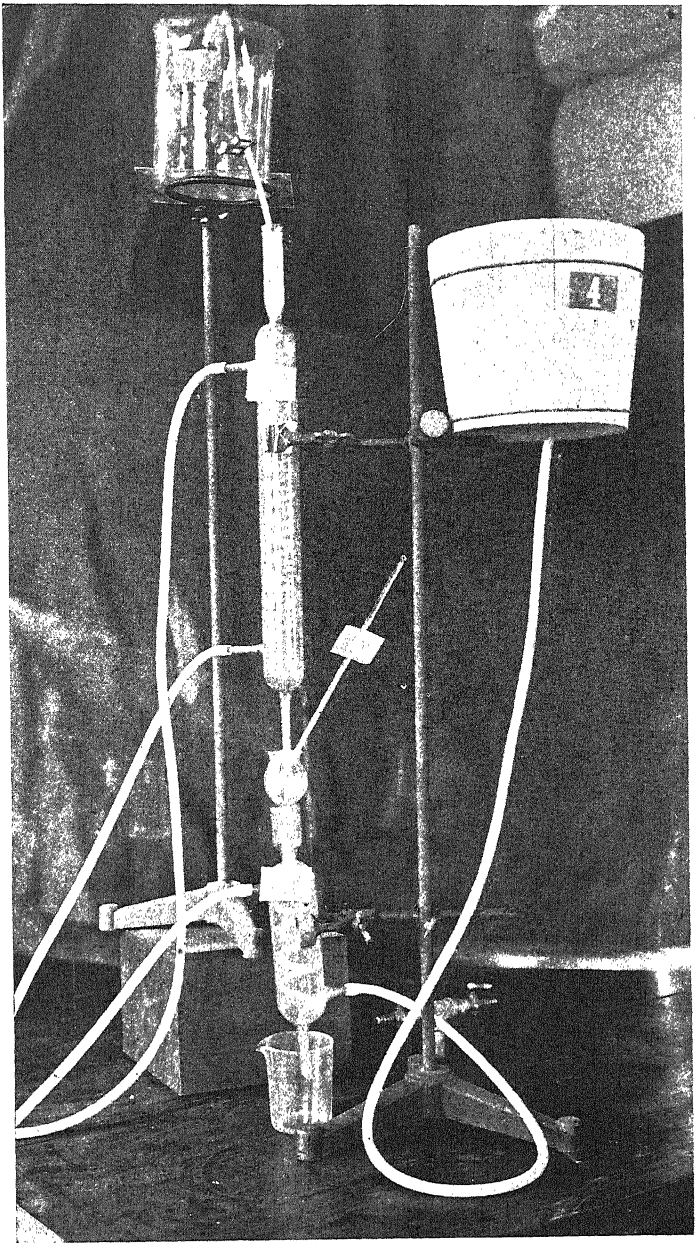


FIG. 2

This work shows that where the regular flash pasteurization temperature of 85°C. is reached, the greater part of the peroxidase is inactivated.

Since inactivation by heat occurs with all enzymes and since this action is thought to be due to an alteration in their general colloidal condition, a condition in which, it is assumed, all enzymes exist, from the foregoing satisfactory agreement of the observed value and those calculated it is to be seen that the rate of inactivation of the enzyme peroxidase on heating follows a definite law. The rate of inactivation is a function of the temperature and time of exposure. It was found that if milk was heated to 62.5°C and held at that temperature for twenty minutes had little effect on the peroxidase. Our results are in close agreement with the findings of Enler and Pape, who heated the juice of fresh horse radish to 60°C. for two hours and found that the activitty of the peroxidase diminished only in the proportion of 7 to 5. We can, therefore, reasonably conclude that the optimal temperature is in the neighborhood of 60°C. and the maximal temperature at which the peroxidase is completely inactivated at 80 to 85°C. For most enzymes the optimal temperature is stated to be between 40 to 55°C. and the maximal temperature to be between 65 to 75°C. It is well known that a rise in temperature causes an increased activity of the enzymes, however, the point where inactivation begins the speed of reaction is retarded. As the temperature is increased the destruction increases more rapidly than the increase in the effect of heat on the acceleration of enzyme action, when the curve begins to drop, and at the maximal temperature the curve ceases to exist. This is true of all enzyme actions. Unfortunately very few enzymes can be studied as critically as the oxidases and peroxidases. From what is known concerning the enzymic action we can briefly state that all enzymes are inactivated at about the same temperature and all enzymes are colloidal and the paralysis by heat seems to be due to an alteration of the colloidal condition, hence, we should expect the same amount of heat to produce a similar effect on all enzymes.

THE INFLUENCE OF CERTAIN FACTORS ON THE HYDROGEN ION CONCENTRATION OF MILK

II. TEMPERATURE CHANGES

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In connection with the acidity studies as effected by feeding (1), it seemed desirable to study the effect of temperature changes. Hence, the following studies were made on milk from the same cows as were used in the other experiments.

The samples (100 cc.), were titrated at room temperature then at higher temperatures, the heating being done by partially immersing a flask containing the sample in hot water and shaking until the milk assumed a temperature near that desired and quickly titrating with 0.1 N NaOH, using phenolphthalein as an indicator.

The pH determinations at increased temperatures were made on the same samples as were used for the primary H^+ ion studies. These samples were warmed by immersing the hydrogen electrode vessel in the water bath and when the desired temperature was reached, the determinations were made as soon as possible, about five minutes being required to bring the electrode to equilibrium. The temperature was taken at the time the determinations were actually made, with both the H^+ ion and total acidity determinations.

From a study of tables 1 and 2 it can be seen that both the H^+ ion concentration (pH) and the total (titratible) acidity of milk increase upon heating, which is contrary to what Van Dam (2) found.

The effect of temperature on the acidity of milk was so marked that it seemed desirable to make further studies on the effect of

TABLE 1

Effect of increase in temperature on the pH value of fresh cow's milk

DATE	COW NUMBER	TEMPERATURE AND pH RANGES				
3/20/22	43	Temperature pH	17° 6.06	25° 5.98		
3/21/22	43	Temperature pH	20° 6.04	44° 5.89	49° 5.80	
3/22/22	43	Temperature pH	19° 6.17	32° 6.15	35° 6.12	50° 5.89
3/20/22	229	Temperature pH	18.5° 6.32	41° 6.05		
3/21/22	229	Temperature pH	22° 6.30	37° 6.18	47° 6.06	
3/20/22	297	Temperature pH	20° 6.24	35° 6.09	40° 6.00	43° 5.93
3/21/22	297	Temperature pH	20° 6.23	56° 5.49		
3/22/22	297	Temperature pH	19° 6.10	43° 6.08	58° 5.87	

TABLE 2

Effect of increase in temperature on the total acidity of cow's milk

DATE	COW NUMBER	TEMPERATURE AND ACIDITY RANGE					
3/20/22	43	Temperature cc. NaOH	21° 23.30	39° 24.40	55° 25.80	73° 26.46	80° 27.76
3/21/22	43	Temperature cc. NaOH	21° 31.70	48° 33.73	65.5° 37.70	72° 42.33	
3/21/22	229	Temperature cc. NaOH	22° 31.45	48° 37.70	65° 40.55	75° 43.75	
3/20/22	297	Temperature cc. NaOH	20° 22.90	45° 24.50	70° 26.45	84° 28.30	
3/21/22	297	Temperature cc. NaOH	20° 25.60	43° 28.75	71° 30.85	77° 33.75	80° 36.45
3/22/22	297	Temperature cc. NaOH	19° 26.90	32° 28.90	40° 30.56	52° 32.25	60° 33.96
3/22/22	297	Temperature cc. NaOH	70° 35.30	80° 38.64			

temperature changes on the total acidity of milk, using both the old method and the new method of Van Slyke and Bosworth (3). (See table 3.)

TABLE 3

Effect of increase in temperature on the total acidity of cow's milk

SAMPLE NUMBER	TEMPERATURE AND ACIDITY RANGE						
I. Old method. Heated with NaOH in milk from previous titrations							
1a	Temperature	17.5°	31.0°	52.0°	68.0°	80.0°	
	cc. NaOH	20.70	21.80	23.52	24.21	25.13	
2a	Temperature	21.0°	46.0°	64.0°	75.0°	80.0°	
	cc. NaOH	21.68	22.80	24.32	25.52	27.70	
II. Van Slyke and Bosworth's New Method, but milk heated as in I							
1a	Temperature	17.5°	32.0	52.0°	Spilled at this point		
	cc. NaOH	7.20	7.70	8.88			
2a	Temperature	19.5°	35.0°	54.0°	70.0°	80.0°	
	cc. NaOH	7.74	8.46	9.60	12.88	15.86	
3a	Temperature	20.0°	35.0°	52.0°	68.0°	78.0°	82.0°
	cc. NaOH	8.00	8.26	8.80	11.22	14.40	16.10
III. Old method. Milk heated in absence of alkali							
1b	Temperature	21.0°	37.0°		57.0°	65.5°	
	cc. NaOH	21.00	21.30		22.70	22.98	
2b	Temperature	22.0°	40.0°	53.0°	69.0°		
	cc. NaOH	20.80	22.16	22.60	23.10		
IV. New method. Milk heated in absence of alkali							
1b	Temperature	21.0°	37.0°	61.0°	66.0°	71.0°	
	cc. NaOH	7.50	7.60	8.58	9.02	9.70	
2b	Temperature	21.0°	42.0°	64.0°	72.0°	75.0°	
	cc. NaOH	7.50	7.80	9.08	9.80	10.08	

Both methods were used under the two conditions mentioned below. Under the first set of conditions the sample from the first titration was warmed for the second titration and after being neutralized by the second titration was again warmed for

the third, and so on for the complete series of titrations on that particular sample. Thus a neutral sample was warmed each time for the succeeding titration.

In the second set of conditions, fresh samples were warmed each time for each succeeding titration.

From table 3 it can be seen that the presence of alkali in the sample when it is heated causes an extra increase in the acidity. When the temperature reaches about 80°C. a brown color develops and a rapid increase in acidity takes place. This is likely due to some lactose being oxidized by the alkali. Only a slight color develops when a sample is heated to 80° in absence of alkali. The effect of the alkali is shown much more when the method of Van Slyke and Bosworth is used.

CONCLUSIONS

The effect of increased temperatures on the hydrogen ion concentration and total acidity of milk was studied. Two methods for total acidity were compared. An increase in temperature caused an increase in both total acidity and acidity as pH value, whether samples previously neutralized or fresh samples were used for the determinations.

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THE RATE OF MILK SECRETION AS AFFECTED BY AN ACCUMULATION OF MILK IN THE MAMMARY GLAND

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Many investigations have shown that the milk of all mammals is a true sectetion. The experimental work on the precursors of the various constituents of milk has been summarized by Meigs (1) and need not be repeated here. If milk is a true secretion, then it seems reasonable to expect that the rate of secretion would follow an orderly course governed by some law of nature. As the transformation of the precursors of milk to the various normal constituents of milk are chemical in nature, the rate of change or the rate of secretion might reasonably be expected to follow the rate of change of certain chemical reactions.

Assuming that milk secretion is chemical in nature, it, like other chemical reactions, is governed by the law of mass action. In other words, the rate of milk secretion is dependent upon the active mass of the precursors of milk in the blood and on the removal of the products of the reaction. In addition, certain harmones or internal secretions may act as an activator or catalyzer.

The object of this paper is to show the effect of an accumulation of milk in the udder on the rate of milk secretion. Kaull (2), in Germany found that when cows were milked at various intervals up to twelve hours the rate of milk secretion was very materially influenced by the period between milkings. Practical dairymen (3) have also observed the advantages of frequent milking.

PLAN OF INVESTIGATION

In this investigation 4 dairy cows (2 Jerseys and 2 Ayrshires) were milked during an experimental period of about three months. This period was divided into alternate three day experimental and three day rest periods. The cows were milked at 5:00 a.m. and 5:00 p.m. daily with the exceptions mentioned in the following paragraph.

TABLE 1

The effect of the interval between milkings on the rate of secretion and composition of milk

HOURS AFTER PRECEDING MILKING	MILK	FAT	FAT	SPECIFIC GRAVITY	SOLIDS
	pounds	*per cent*	*pounds*		*per cent*
1	1.05	7.56	0.084		
2	1.51	7.76	0.123		
3	2.61	6.94	0.185	1.0302	15.60
4	3.14	6.34	0.200	1.0304	15.38
5	4.28	6.01	0.267	1.0289	15.15
6	4.29	5.94	0.268	1.0292	16.19
7	5.71	5.35	0.322	1.0278	14.87
8	6.39	5.16	0.332	1.0291	14.39
9	6.63	5.37	0.372	1.0280	14.45
10	8.12	4.86	0.418	1.0286	13.91
11	8.96	4.73	0.438	1.0281	13.87
12	8.70	4.63	0.403		
13	10.22	4.15	0.456	1.0284	13.02
14	9.95	3.88	0.404	1.0290	12.70
16	10.60	3.75	0.422	1.0289	
18	11.60	4.24	0.535		
20	12.70	4.13	0.571		
24	13.40	4.59	0.659	1.0304	13.90
28	14.70	4.63	0.759		
32	15.40	3.59	0.597		11.51
36	14.60	3.80	0.685		12.33

During the first three day experimental period an additional milking was made at 6:00 a.m.—one hour after the preceding (5:00 a.m.) milking. In this way the milk secreted during the "first hour" was obtained. During the second three day experimental period the additional milking was made at 7:00 a.m., or two hours after the previous milking. This gave the milk secreted during a two hour period. When the lapse of time after

the last (5:00 a.m.) milking exceeded twelve hours the 5:00 p.m. milking was omitted and when the time exceeded twenty-four hours the following 5:00 a.m. milking was also omitted. In this way the milk secreted at intervals ranging from one to thirty-six hours was secured.

RESULTS OF INVESTIGATION

The averaged data for the 4 cows is shown in figure 1. It will be seen that the greater the amount of milk accumulated in the udder, or the longer the interval between milkings, the less the speed of milk secretion in unit time. This curve has the same shape as the curves representing the rate of change of a reaction in chemistry when the products of the reaction are not removed. This would indicate that milk secretion is indeed a chemical process and follows the usual laws of chemical reactions.

It should be pointed out that there is an error involved in the method of securing the data due to the advance of the stage of lactation in the three month period covered by the investigation. The advance of the stage of lactation and the experimental procedure actually decreased the production of milk to an average of 73 per cent of that produced at the start of the work. Thus the milk secreted toward the end of the experimental period, or for the longer time intervals, may have been slightly less than it would have been had it been possible to conduct the entire investigation at the same time.

The declining curve in figure 1 represents the milk secreted per hour at successive intervals and shows very clearly the effect of the accumulation of milk in the udder on the rate at which milk was secreted. In fact there is a definite rate at which the accumulation of milk inhibits further secretion. Considering the production of milk during the first hour as 100 per cent the rate of the secretion of milk during each succeeding hour is approximately 95 per cent of that of the preceding hour.

This data also throws light on the practical question of the value of milking cows three or four times daily. By referring again to figure 1 it will be noted that during a six-hour period 5 pounds of

milk was secreted, during an eight-hour period 6.3 pounds of milk, and during a twelve-hour period, 8.6 pounds of milk. On a twenty-four hour basis the production of milk during these periods would be as follows:

Two times per day = 8.6 × 2 = 17.2 pounds
Three times per day = 6.3 × 3 = 18.9 pounds
Four times per day = 5.0 × 4 = 20.0 pounds

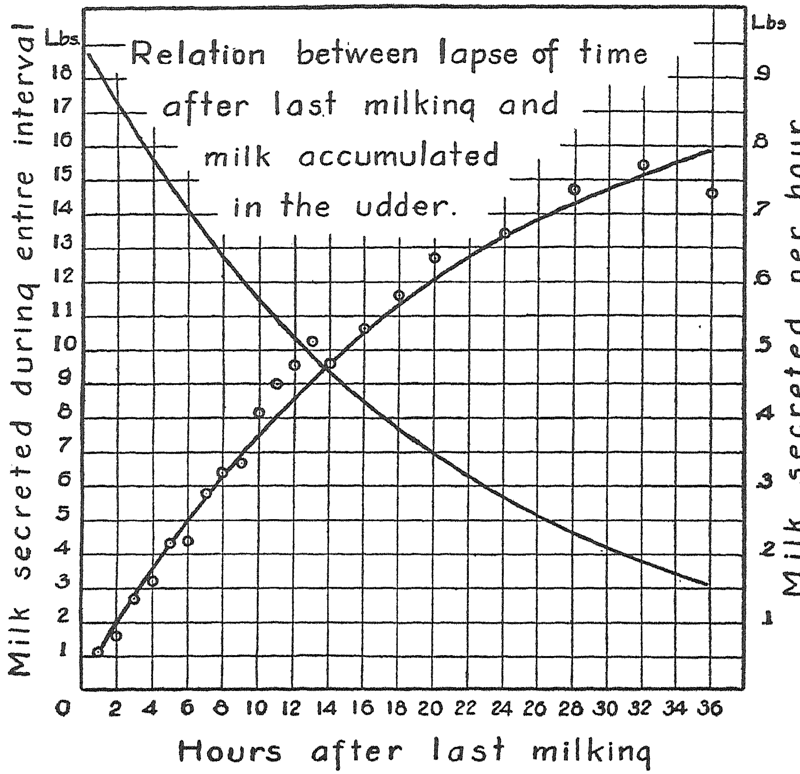

FIG. 1. THE RISING CURVE REPRESENTS THE TOTAL MILK OBTAINED AT THE END OF THE TIME INTERVAL SHOWN IN THE ABSCISSAE

The declining curve represents the milk secreted per hour at successive intervals. Each hour's milk production is shown to be 95 per cent of the production for the preceding hour.

On a percentage basis, considering the production of the cows milk twice daily as 100 per cent, the cows milked three times per day produce 110 per cent, and those milked four times per day

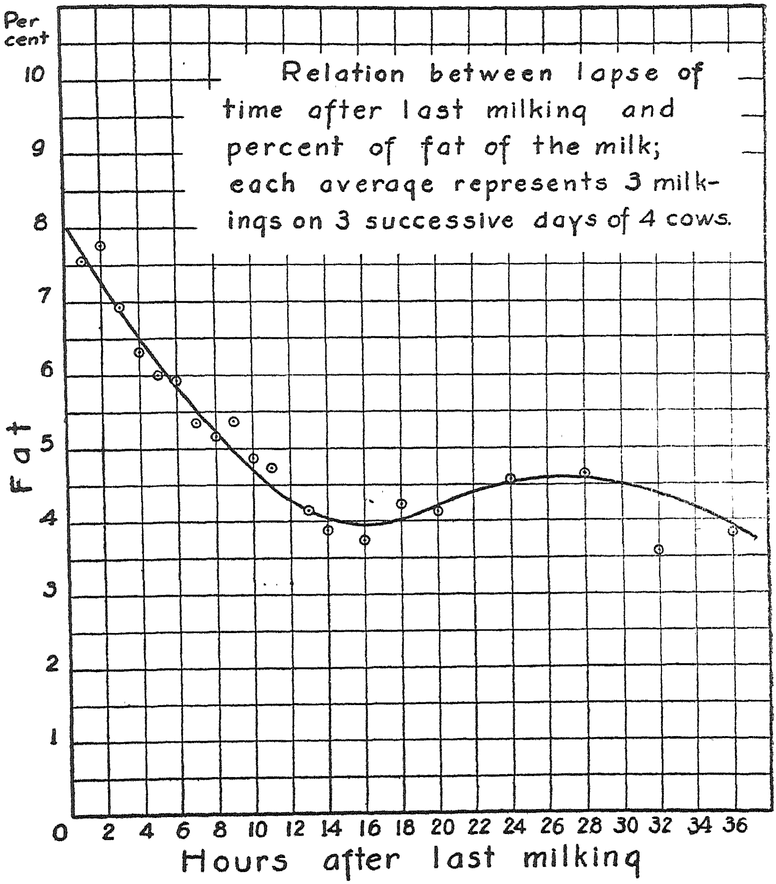

FIG. 2. THE CURVE SHOWS A GRADUAL DECREASE IN THE PER CENT OF FAT IN THE MILK AT VARYING TIME INTERVALS FOLLOWING THE PRECEDING MILKING

116 per cent. It should be noted that this data is based on Jersey and Ayrshire cows averaging less than 20 pounds of milk daily.

The effect of the length of the period between milkings on the composition of the milk is shown in figure 2. The per cent of fat gradually decreases as the interval between milkings increases. The accumulation of milk or the "back pressure" produced from the data here presented, appears to change the relative speed of secretion or resorption of the several milk constituents. However, other data being accumulate at this station seems to indicate a relation between the per cent of fat in milk on the lapse of time after feeding. This rather than the length of time after milking may explain the variation in the composition of milk.

SUMMARY

1. It was shown that the speed of milk secretion in unit time is governed by the amount of milk accumulated in the udder or the interval between milkings. If the amount of milk secreted during the first hour is called 100 per cent the amount of milk secreted each succeeding hour is approximately 95 per cent of that secreted during the preceding hour.

2. The curves showing the rate of milk secretion follow the same course as a chemical reaction when the products of the reaction are not removed indicating that milk secretion is a chemical process and follows the usual laws of physical chemistry.

3. Under the experimental conditions noted it was calculated that cows milked three times per day would produce 110 per cent and those milked four times per day 116 per cent of the milk secreted by cows milked twice daily.

4. The data indicates that the per cent of fat and total solids gradually decrease with the lengthening of the interval between milkings until the time interval exceeds fourteen to sixteen hours. Thereafter there is a slight increase up until the twenty-fourth to twenty-sixth hour, followed again by a gradual decline until the thirty-sixth hour.

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THE INFLUENCE OF THE STAGE OF LACTATION ON THE PRODUCTION OF DAIRY COWS

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In determining the real producing ability of a dairy cow it must be remembered that phenomenal milk production for a short time and then a rapid decrease does not lead to greatest or most economical milk production. Too often persistency of production has been forgotten and emphasis laid only on the production of a cow during the early portion of her lactation period.

RÉSUMÉ OF PREVIOUS WORK

A number of references are to be found on the production of cows for but a short time but these will be looked on as inapt for insertion here. Three separate workers, Linfield (2), Woll (3) and Grady (1), have worked with a combined total of several hundred cows of the Holstein, Guernsey and Jersey breeds. The average of their results shows that there is a slow but steady decrease from the first month of the lactation to about the seventh month and then the decrease becomes more rapid until the cows are dry.

DATA PRESENTED

The entire material presented here has been collected from the herd on the Iowa State College Dairy Farm, from December, 1907, to December, 1921. All normal lactations have been considered and a normal lactation has been looked on as not exceeding in duration 12 consecutive periods of 30 days each. Where a lactation exceeded 360 days the extra days were thrown off, but if the cow dried off naturally before the expiration of 360 days, the lactation was included as normal for the cow at that time.

The greater portion of the data has been obtained from the purebred herd but additional records were obtained from an experimental herd of scrub cows and the two generations out of them and by purebred bulls which have already completed records. On the whole 428 lactations of 162 cows have been used. No correction for age or other factors has been made.

The average production of the Ayrshire, Guernsey, Holstein and Jersey breeds have been tabulated separately by months and in a similar tabulation are included the scrubs, first grades, second grades and all the purebreds for comparative purposes.

TABLE 1

Summary of data used

BREED	NUMBER OF COWS	NUMBER OF LACTATIONS
Ayrshire	12	29
Guernsey	26	81
Holstein	30	82
Jersey	40	108
All purebreds	116	300
Scrubs	14	57
First grades	14	38
Second grades	16	33
All scrubs and grades	46	128
Total	162	428

From these it is evident that there is a general decrease in milk and fat production from the first to the last month of the lactation, although in the cases of the Ayrshires and Holsteins the highest level of milk production is reached in the second period. When all the purebreds are grouped together this is also found to be true.

In the percentage of fat in the milk the Jerseys show a gradual rise from the beginning to the end of the lactation period, while the Guernseys show a drop in the second month only and then the percentage of fat rises until the end of the lactation. In the case of each of these breeds the average fat percentage for the

TABLE 2

Average production of purebreds by breeds

MONTH	AYRSHIRE			GUERNSEY			HOLSTEIN			JERSEY		
	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content
	pounds	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*
1	951.5	40.58	4.26	831.8	38.23	4.60	1353.7	49.27	3.64	769.4	35.97	4.67
2	992.0	37.76	3.81	822.8	36.58	4.45	1401.3	46.36	3.31	755.8	35.52	4.70
3	923.5	34.85	3.77	749.2	33.82	4.51	1302.1	41.91	3.22	693.3	32.93	4.75
4	831.9	31.34	3.77	669.2	30.84	4.61	1192.5	39.08	3.28	626.3	30.68	4.90
5	752.5	28.94	3.85	601.1	28.41	4.73	1073.6	35.01	3.26	573.7	28.70	5.00
6	703.7	27.40	3.89	559.7	26.59	4.75	984.9	32.64	3.31	516.8	26.33	5.09
7	654.9	25.50	3.89	515.6	24.74	4.80	912.5	30.47	3.34	475.3	24.39	5.13
8	603.7	23.59	3.91	461.4	22.77	4.93	850.2	28.87	3.40	433.5	22.29	5.14
9	546.3	21.48	3.93	410.2	20.64	5.03	766.7	26.21	3.42	380.5	19.94	5.24
10	436.8	17.79	4.07	346.4	17.51	5.05	665.8	22.88	3.44	330.7	17.59	5.32
11	312.3	13.12	4.20	285.8	14.83	5.19	583.6	20.53	3.52	272.2	14.61	5.37
12	222.9	9.47	4.25	242.3	12.72	5.25	474.7	17.09	3.60	224.8	12.07	5.37
Year..	7932.0	311.82	3.93	6495.5	307.68	4.74	11561.6	390.32	3.38	6052.3	301.02	4.97

TABLE 3

Average production of scrubs, grades and purebreds

MONTH	SCRUBS			FIRST GRADES			SECOND GRADES			ALL PUREBREDS		
	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content
	pounds	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*
1	610.5	28.73	4.71	901.5	40.15	4.45	809.9	35.88	4.43	963.6	40.65	4.32
2	572.0	26.63	4.64	827.6	36.72	4.44	817.3	34.71	4.25	973.2	38.96	4.00
3	487.7	22.81	4.68	744.3	33.43	4.49	762.1	32.29	4.24	897.0	35.81	3.99
4	429.8	19.90	4.63	653.9	29.87	4.57	691.0	29.98	4.34	812.5	33.08	4.07
5	360.6	16.72	4.63	576.4	26.59	4.61	653.7	28.63	4.38	735.0	30.37	4.13
6	295.7	13.87	4.69	519.0	24.34	4.69	619.4	27.80	4.49	674.4	28.23	4.19
7	246.5	11.66	4.73	465.8	21.97	4.72	570.0	25.97	4.56	623.0	26.29	4.22
8	207.9	10.15	4.88	419.0	20.06	4.79	548.3	25.26	4.60	571.4	24.35	4.26
9	159.7	8.02	5.02	366.7	17.97	4.90	501.5	24.08	4.80	510.1	21.99	4.31
10	103.8	5.39	5.02	285.6	14.30	5.01	455.9	21.43	4.70	436.8	19.03	4.36
11	56.9	3.05	5.36	202.7	10.61	5.23	388.1	18.58	4.79	364.9	16.14	4.42
12	28.0	1.52	5.43	121.9	6.68	5.48	327.3	15.54	4.75	297.7	13.37	4.54
Year..	3559.1	168.46	4.73	6084.4	282.69	4.65	7144.5	320.15	4.48	7859.6	328.27	4.18

last month of the lactation and the average fat percentage for the whole year are higher than the average fat percentage for the first month of the lactation.

In the case of the Ayrshires there is a drop in fat content to the third month and this is maintained through the fourth. From then on the fat content increases fairly constantly. The

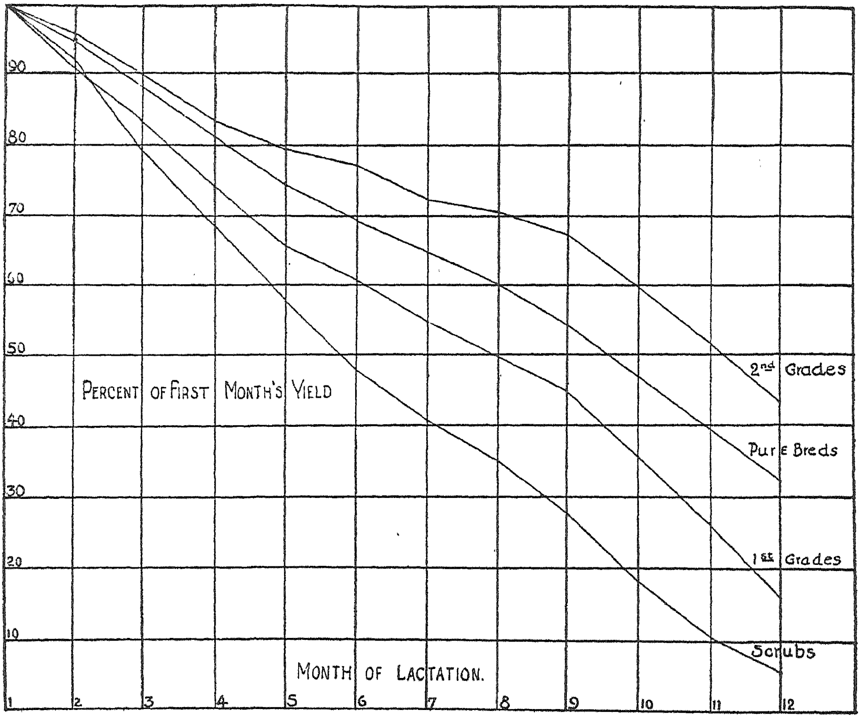

Chart 1. Relative Persistency in Fat Production of Scrub, Grade and Purebred Cows

Holsteins show a drop in fat content to the third month with a rise from then to the end of the lactation period with but one insignificant change. In the case of both the Ayrshires and Holsteins the fat content of the first month's milk is apparently high and this level is not again reached until the twelfth month. Consequently, the average fat percentage for the year for each breed is lower than that for the first month of the lactation.

TABLE 4

Monthly production expressed as a percentage of the first month's production for the purebreds by breeds

MONTH	AYRSHIRE			GUERNSEY			HOLSTEIN			JERSEY		
	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	100	100	100	100	100	100	100	100	100	100	100	100
2	104	93	89	99	96	97	104	94	91	98	99	100
3	97	86	88	90	88	98	96	85	89	90	92	102
4	87	77	88	80	81	100	88	79	90	81	85	105
5	79	71	90	72	74	103	79	71	90	75	80	107
6	74	68	91	67	70	103	73	66	91	67	73	109
7	69	63	91	61	65	104	67	62	92	62	68	110
8	63	58	92	55	60	107	63	59	93	56	62	110
9	57	53	92	49	54	109	57	53	94	49	55	112
10	46	44	96	42	46	114	49	46	95	43	49	114
11	33	32	99	34	39	113	43	42	97	35	41	115
12	23	23	100	30	33	114	35	35	99	29	34	115
Year...	834	768	92	781	805	103	854	792	93	781	837	106

TABLE 5

Monthly production expressed as a percentage of the first production for scrubs, grades and purebreds

MONTH	SCRUBS			FIRST GRADES			SECOND GRADES			ALL PUREBREDS		
	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content	Milk yield	Fat yield	Fat content
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	100	100	100	100	100	100	100	100	100	100	100	100
2	94	93	99	92	92	100	101	97	96	101	96	95
3	80	79	99	83	83	101	94	90	96	93	88	95
4	70	69	98	73	74	103	85	84	98	84	81	97
5	59	58	98	64	66	104	81	80	99	76	75	98
6	49	48	100	58	61	105	77	78	101	70	69	99
7	40	41	100	52	55	106	70	72	103	65	65	100
8	34	35	104	47	50	108	68	70	104	59	60	101
9	26	28	107	41	45	110	62	67	108	53	54	102
10	17	19	107	32	36	113	56	60	106	45	47	103
11	9	11	114	23	26	118	48	52	108	38	40	105
12	5	5	115	14	17	123	40	43	107	31	33	106
Year...	583	586	100	675	704	105	882	892	101	876	808	99

From the summaries of the records prepared the production in the first month has been taken as 100 and the production in each succeeding month expressed as a percentage of the production of the first month. This gives a record of the persistency of production of the various breeds and grades. From this it is evident that those producing well in the first month do not always give the highest production for the year as their decrease in production may be rapid.

It will be found from chart 1 where fat production alone is considered that the scrubs declined most rapidly in production and their first month's production is also the lowest so they were the poorest producers. The first grades had a higher production during the first month than did the second grades but their decline in production was comparatively rapid and so they did not have as high an annual production as did the second generation of grades. On the other hand the second generation of grades did not have as high initial production as the purebreds and even though they did show remarkable persistency in production yet the average record of these individuals was not as great as the average for the pure purebreds.

SUMMARY

From the data presented here it would appear that:

1. High production at the beginning of the lactation period is not the only factor of importance in determining the production of a cow.
2. Persistency of production, coupled with high initial production, will lead to good production.
3. There is a general downward trend in milk and butterfat production from the beginning to the end of the lactation and with this would appear to go for the first month or two a decrease and later an increase in the fat content of the milk. This may be generally attributed to the fact that changes in the yield of fat, follow, but lag behind, the changes in the yield of milk.
4. Holsteins and Ayrshires showed the highest milk production in the second month and all others in the first month of the lactation.

5. In the case of the Jerseys the percentage of fat in the milk rises from the beginning to the end of the lactation period, while the Guernseys show a drop in the second month only. In both breeds the average fat percentage for the year is greater than the average for the first month.

6. The Ayrshires and Holsteins produce milk of decreasing fat content for some time after freshening and the average fat content of the milk for the year is less than for the first month.

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RELATION OF MILK PRODUCTION TO THE TWINNING TENDENCY[1]

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Upon first thought it seems unreasonable to think of any relation between high milk production and multiple births in cattle. However, when we reflect that twinning in cattle is but one of the evidences of high fertility and that high fertility has been found to be closely correlated with high milk production (1), such a relationship does not seem so unreasonable. The writer, in order to determine whether or not such a relationship does exist has made a study of a random sample of 357 dams of multiple offspring in the Holstein-Friesian breed.

In this study are included the dams of all the male twins in volume 35 of the herd-book, and the dams of all the female twins indicated in volume 40, up to number 474,273. There were a total, then, of 165 dams of male twins or male and freemartin, and a total of 192 dams of female twins. The two groups were kept separate as the males are selected more rigorously than the females, and thus the dams of registered males are more likely to have records than the dams of registered females. The A. R. O. records of the dams of the twins were compiled, the problem being, "Do the dams of twins produce more milk than the dams of singles." Of course, if one group did produce more than the other, it would be indicated, not by a higher average A. R. O. production, but by a larger number of animals making the A. R. O. requirements. The table given below shows the results of the study:

[1] A part of a thesis, Twinning in Dairy Cattle, submitted in partial fulfillment of the requirements for graduation.

	per cent
Percentage of dams (of twins indicated in the list of males) having records	55.7
Percentage of dams (of twins indicated in the list of females) having records	45.8
Percentage of all registered females having records[2]	20.4

According to the above table, then, the dam of twins has more than twice as good a chance to make the A. R. O. requirements as the ordinary female. However, right here an error enters in. We are comparing a selected group of females (mature cows that have produced living offspring) with the whole group of females. The question which enters in is, how many females, once registered, fail to produce living offspring? The writer could get no definite data with which to correct the error, but did get the opinions of several well informed men upon the question. Their estimates varied from 2 to 12 per cent. Using the highest figure which the reader will admit seems rather high, the percentages remain 32.4 per cent of all dams making the A. R. O. and 55.7 and 45.8 per cent respectively for the dams of twins.

It is interesting to note in the consideration of this question that 82.7 per cent of the sires of the dams of twins in this list had at least one daughter in the advanced registry. The average number of daughters for those sires of twin producers having daughters in the advanced registry was over sixteen. The reader may use his own judgment as to whether or not this seems higher than in the average pedigree.

Another important fact is that great animals such as century sires recur very often in the pedigrees of twins. This is true of all pedigrees because of constant selection, but is it true to the same extent? For example, Hengerveld DeKol 23102 occurs as the grandsire of 5 per cent of the twin producers whose twins are registered in volume 35 of the herd-book. If he occurred in the same proportion for all animals he must needs have 750

[2] The writer judged that all females registered before August 17, 1918, had had an opportunity to make the A. R. O. requirements before the 1922 Advanced Registry Yearbook was published. While some will make records that have not already done so, it was thought that an equal number registered since that date have made records.

Sarcastic Lad 23971

Johanna Aaggies Sarcastic Lad
26935

Aaggie Cornucopia Johanna Lad
32554

Johanna Aaggie

Aaggie Cornucopia Pauline
48426

Aaggie Cornucopia Johanna Lad 11th
87681

Edith Grace Piebe
82626

Sarcastic Lad 23971

Johanna Sarcastic Cornucopia (Twin)
261769
Also the dam of Twins

Johanna Aaggie's Sarcastic Lad
26935

Johanna Aaggie

Lileth Paul DeKol's Son
30412

Sir Grace DeKol Joh. Paul
35408

Lileth Pauline DeKol

Otego Valley Sarcastic
125547

Grace DeKol 2d
54789

Sarcastic Lad 23971

Johanna Aaggie's Sarcastic Lad
26935

Johanna Aaggie

Madlinette Sarcastic
80265

Catrina 5th's Pieterje
39113

Hengerveld DeKol
23102

Sarcastic Lad
23971

Hengerveld DeKol 5th (Twin)
54721

Joh. Rue 3d's Lad
26934

Belle Neth. Joh
62304

Johanna Rue 3d

Marcella Hengerveld DeKol
70519

Belle Neth. Clothilde
33836

Marcella Lote 66934

Grace Darling Hengerveld.
242862
The dam of five sets of
Twins

Sarcastic Lad
23971

Tina Clay DeKol Lad
42450

Tina Clay DeKol
57350

Grace Darling of Wooster
242861
Dam of nine singles only

Sarcastic Lad
23971

Tina Clay DeKol Lad
42450

Grace Daw 2d
242860
Dam of ten singles and
Twins

Tina Clay DeKol
57350

Grace Daw 64996

granddaughters having *sons* registered in the single herdbook volume 35 which contains registrations for six and one-half months. Such a performance is, of course, rather doubtful. Similar examples of Pontiac Korndyke, King of the Pontiacs, King Segis, King Korndyke Manor DeKol and others might be pointed out. Sarcastic Lad 23971 seems to be a carrier of the twinning tendency. Two pedigrees of very fertile families are given herewith showing the relation of the name of Sarcastic Lad to twinning. Pedigrees of a similar nature showing the occurrence of the other great bulls named above in relation to twinning might also be given.

Objection has been made to the theme of this article upon the grounds that world's records have not been made by twins. In answer it is interesting to point out that the world's record milk production for a junior-two-year-old was recently broken by a freemartin, Woodbine Rosa Prilly, in California. She carried calf five months and gained several hundred pounds in weight during the test. Also the record of Pontiac Clothilde DeKol 2d, a dam of twins, who produced 1017.28 pounds of fat as early as 1910. Grace Darling Hengerveld, who calved five sets of twins, also produced (unofficial) 21,528 pounds of milk and 1023 pounds of fat in a single milking period of 658 days.

It is to be admitted that the above discussion does not include enough numbers nor sufficient data to furnish absolute proof. It is submitted in the hope that interest in the problem may be stimulated and that in the end the dairy industry may benefit. Conclusions that may be drawn from the limited data at hand are:

1. According to authority, there is a definite relationship between high fertility and high milk production, and partial sterility and low milk production.

2. This relationship seems to hold true when high fertility is evidenced in the production of twins.

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THE EFFECT OF AN INCOMPLETE REMOVAL OF MILK FROM THE UDDER ON THE QUANTITY AND COMPOSITION OF THE MILK PRODUCED DURING THE IMMEDIATE SUBSEQUENT MILKINGS

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The necessity of a preliminary milking as required by some of the pure bred dairy cattle clubs in advanced registry testing has caused a great deal of discussion of late among the dairy cattle clubs and the agricultural colleges and experiment stations supervising advanced registry testing. If no preliminary milking is taken, i.e., if the supervisor in charge of the test does not see that the cow is milked dry at the milking previous to the starting of the test, it is possible for the milker to leave a portion of the milk in the cow's udder and by so doing change the quantity and composition of the milk produced at the succeeding milkings. Such a change in the composition of the milk produced has been demonstrated by Regan and Mead (1) and Fitch, Becker and McGilliard (2) and the proof of this change based upon an observed increase in the percentage fat content and quantity of milk produced. The percentage fat content of milk, however, is extremely variable from one milking to another, and may be influenced to a great extent by factors other than the leaving of a portion of the milk within the udder.[1] On the other hand, Jackson and Rothera (3) have shown that certain constituents of milk (percentage lactose and ash contents) which under normal conditions are approximately constant from one milking to another, when once disturbed show a marked variation and return to their original condition very slowly. This variation in the percentage lactose and ash contents was produced by returning a portion of the milk to

[1] The influence of such factors upon the percentage fat content of milk has been demonstrated by Anderson (4) Rasmussen (5) and Armsby (6)

the udder after it was milked dry. During the immediate subsequent milkings the percentage lactose content was markedly decreased and the percentage ash content similarly increased.

In an investigation by the author wherein a portion of the milk was returned to the udder after a dry milking, it was found that

TABLE A

Effect of returning milk to the udder

Cow 276, pure bred Jersey; age three years seven months; calved August 24, 1922; milked two times per day, September 25, p.m. to October 4, p.m., 1922.

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	1.7	4.0	0.068	5.33	0.091	4.48	0.076	13.81	0.235
	2.5	4.45	0.111	5.20	0.130	4.03	0.101	13.68	0.342
	2.4	6.2	0.149	5.50	0.132	3.78	0.091	15.48	0.372
	2.3	4.6	0.106	5.29	0.122	3.76	0.086	13.65	0.314
	2.1	4.9	0.103	5.25	0.110	3.94	0.083	14.09	0.296
	2.3	4.1	0.094	5.30	0.122	3.84	0.088	13.24	0.305
	2.0	4.1	0.082	5.33	0.107	4.17	0.083	13.60	0.272
	2.3	5.0	0.115	5.29	0.122	3.83	0.088	14.12	0.325
	1.9	4.35	0.083	5.24	0.100	3.91	0.074	13.50	0.257
	2.2	5.1	0.112	5.18	0.114	4.11	0.090	14.39	0.317
Milk returned	1.5	2.65	0.040	5.27	0.079	4.07	0.061	11.99	0.180
Subsequent milkings	2.8	5.20	0.146	2.54	0.071	4.94	0.138	12.68	0.355
	0.4	9.10	0.036	3.71	0.015	6.35	0.025	19.16	0.077
	1.4	5.25	0.074	3.66	0.051	6.14	0.086	15.05	0.211
	1.1	4.20	0.046	4.45	0.049	4.86	0.053	13.51	0.149
Average preliminary milkings	2.17	4.71	0.102	5.30	0.115	3.96	0.086	13.99	0.304
Average subsequent milkings	1.43	5.30	0.076	3.26	0.047	5.30	0.076	13.90	0.198

during the immediate subsequent milkings the percentage lactose content was markedly decreased and the percentage protein + ash content similarly increased. Table A and figure A give the results from one of a series of trials all of which gave corresponding results.

The figures in this table represent the production for one-quarter of the udder. Approximately 0.66 pound of milk was returned

to the quarter. Bacterial analysis showed that the milk returned to the quarter was practically free from bacteria.

From figure A it is quite evident that during the subsequent milking the percentage lactose and protein + ash contents markedly deviate from their normal condition and then return to it very slowly. The percentage fat content shows a marked

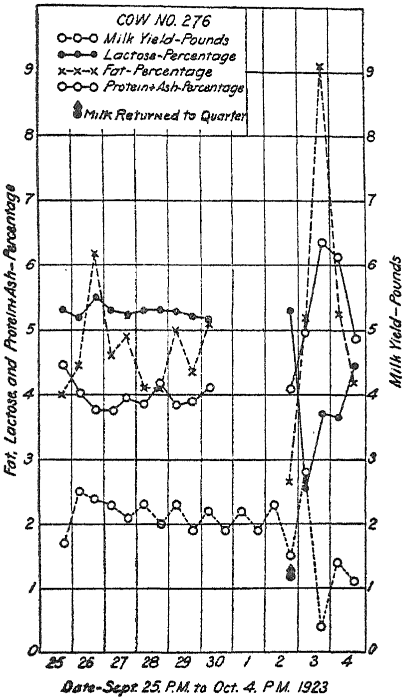


Fig. A.

increase, the greatest increase generally falling on the second milking after the return of milk to the udder. It might be well to point out at this time that such a manipulation could be carried on in advanced registry testing and the percentage fat content greatly increased by returning only a small amount of milk to the udder *shortly* after it was milked dry.

Although the returning of a certain portion of milk to the udder is not the same as leaving a similar portion within the udder by

means of an incomplete milking, it was supposed that the effects produced would be somewhat of the same nature. Futhermore, in case this latter relation held true for all cows, it would be possible by means of a sugar determination of the milk produced at each milking during a two-day test, to detect with relative certainty an incomplete milking any time during the test or within the first two or three milkings previous to the starting of the test. In view of this fact and the bearing it would have upon the requirement of a preliminary milking in advanced registry testing the following experiment was planned.

OUTLINE OF EXPERIMENT

The experiment covered a period of four months—from March 26 to July 27, 1923—and involved the use of 12 pure bred cows in the college herd; viz., 5 Holsteins, 3 Jerseys, 3 Guernseys, and 1 Ayrshire. The cows varied in lactation from one to nine months and ranged from two years eleven months to twelve years of age. The entire experimental period was divided into 10 sub-periods or trials. Particular attention was given to the environment of the animals in order to prevent any disturbance to their production other than that caused by the manipulation.[2]

The first trial involved the use of 4 cows, one of each breed, milked twice a day, and kept under ordinary herd conditions. The cows were milked regularly at 5 a.m. in the morning and 5 p.m. in the evening. The milk was weighed and sampled at each milking and each sample was analyzed for the percentage of fat, lactose, and total solids, the percentage of protein + ash being determined by difference. During this trial 19 milkings were weighed and sampled. The first 8 of the 19 milkings were considered as a preliminary period. At the 15th milking one-fourth of the average milk yield, as computed from the 7 previous corresponding milkings, was left in the udder. The 4 milkings succeeding the manipulation were regarded as comparable to a semi-official two-day test and their average compared to the average of the 8 preliminary milkings.

[2] The leaving of the milk within the udder by means of an incomplete milking will hereinafter be designated as manipulation.

The procedure in the second trial was identical to that in the first trial with the exception that it included only 17 milkings, the first 8 of which were considered as a preliminary period. At the 13th milking one-half of the average milk yield as computed from the 6 previous corresponding milkings, was left in the udder. The Jersey cow 289 used in the first trial was replaced by a Holstein cow 288 because of the Jersey being too far advanced in her lactation. The Guernsey cow 278 went off feed and her record had to be discarded, making a total of only 3 complete records in this trial.

After completing the first two trials with cows milked twice a day, it was decided to continue the experiment with cows on official test, milking 4 times a day. A series of 8 trials then followed, each trial involving the use of one cow and covering a period of four days. Seven cows were used during these trials, 3 Holsteins, 2 Jerseys, and 2 Guernseys, one Guernsey being run twice. Each trial included 17 milkings, all of which were individually weighed and sampled. Each sample, within twenty-four hours after it was taken, was analyzed for the percentage of fat, lactose and total solids, the percentage of protein + ash being determined by difference. The first 8 of the 17 milkings were regarded as a two-day preliminary period. At the 9th milking one-half of the average milk yield, as computed from the 4 previous p.m. milkings, was left in the udder. The 8 milkings subsequent to the manipulation were regarded as comparable to a semi-official two-day test and compared with the 8 preliminary milkings.

In the first two trials the percentage of fat was determined by the Babcock method, the percentage of lactose by the polariscope method, and the percentage of total solids by the gravimetric method. The results obtained from the first two trials indicated that the Babcock method, for determining the percentage of butterfat in milk, was very susceptible to experimental errors which made it rather difficult to accurately determine small differences in percentage. In view of this fact and the necessity for the most accurate results, the Babcock method in trials III to X was replaced by the Roese-Gottlieb method, the percentages of lactose, total solids and protein + ash being determined the same as in

trials I and II. During the preliminary milkings of each trial the front quarters of the udder were milked as one unit and likewise the rear quarters, and the milk weights recorded separately for the respective pairs. Such a procedure was necessary in order to insure an even distribution of milk between the quarters of the udder when the udder was not milked dry.

EXPERIMENTAL RESULTS

The results from the various trials are reported in tables 1 to 15 and figures 1 to 15. Each table and figure represents the production of one cow. Although the results in tables 1 to 15 are in very few cases as striking as those in table A, there is a general tendency towards the latter. The average increases and decreases in the percentage fat, lactose and protein + ash contents and the yields of fat and milk, as determined from the preliminary and subsequent milkings, were in most cases not very great, and it was necessary to set a standard below which differences were not considered as significant.[3] In very few cases do the graphs in figures 1 to 15 show as marked a disturbance in the various percentage contents and yields as do the graphs in figure A.

Tables 1 to 4 and figures 1 to 4 include the results of the first trial. The cows were milked twice a day during this trial and one-fourth of the average milk yield was left in the udder. Of the 4 cows tested, one showed a significant average increase in the yield of milk and 2 a significant average increase in both the percentage fat content and the yield of fat. Three of the four cows showed a significant average decrease in the percentage lactose content and two a significant average increase in the percentage protein + ash content. The Jersey cow 289 was the only cow to show significant differences in the percentage fat, lactose and protein + ash contents similar to those found in table A. The Ayrshire cow 277 was the only cow to show no significant differences in any of the above mentioned percentage contents and yields.

[3] A significant average increase or decrease was considered as not less than 0.1 for percentage content, 0.71 pound for milk yield, and 0.03 pound for fat yield.

TABLE 1

Effect of an incomplete milking

Cow 200, pure bred Holstein; age 10 years 2 months; calved August 27, 1922; milked 2 times per day; March 26, p.m. to April 6, p.m. 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	15.8	3.20	0.506	4.69	0.741	3.52	0.556	11.41	1.803
	18.5	3.60	0.666	4.77	0.882	3.65	0.675	12.02	2.224
	17.3	3.35	0.580	4.78	0.827	3.29	0.569	11.42	1.976
	18.5	3.60	0.666	4.77	0.882	3.44	0.636	11.81	2.185
	18.2	3.40	0.619	4.57	0.832	3.69	0.672	11.66	2.122
	17.2	3.60	0.619	4.73	0.814	3.64	0.626	11.97	2.059
	18.0	3.45	0.621	4.55	0.819	3.61	0.650	11.61	2.090
	17.6	3.75	0.660	4.69	0.825	3.45	0.607	11.89	2.093
Incomplete milking	13.0	2.60	0.338	4.36	0.567	3.62	0.471	10.58	1.375
Subsequent milkings	18.9	3.70	0.699	4.10	0.775	3.97	0.750	11.77	2.225
	15.3	3.65	0.558	4.50	0.689	3.56	0.545	11.71	1.792
	17.6	3.45	0.607	4.58	0.806	3.48	0.612	11.51	2.026
	16.9	3.50	0.592	4.54	0.767	3.52	0.595	11.56	1.954
Average preliminary milkings	17.64	3.50	0.617	4.69	0.823	3.54	0.624	11.73	2.069
Average subsequent milkings	17.18	3.58	0.614	4.42	0.759	3.64	0.625	11.64	1.999

TABLE 2

Effect of an incomplete milking

Cow 289, pure bred Jersey; age seven years two months; calved May 21, 1922; milked two times per day; March 26, p.m. to April 6, p.m. 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	7.5	6.45	0.484	5.30	0.398	4.70	0.353	16.45	1.234
	5.5	5.15	0.283	5.57	0.306	4.70	0.259	15.42	0.848
	7.4	6.55	0.485	5.33	0.394	4.51	0.334	16.39	1.213
	6.0	5.55	0.333	5.57	0.334	4.56	0.274	15.68	0.941
	7.3	6.05	0.442	5.31	0.388	4.51	0.329	15.87	1.159
	6.7	6.60	0.442	5.33	0.357	4.63	0.310	16.56	1.110
	6.6	5.55	0.366	5.26	0.347	4.83	0.319	15.64	1.032
	7.1	6.30	0.447	5.49	0.390	4.62	0.328	16.41	1.165
Incomplete milking	5.2	4.35	0.226	5.29	0.275	4.75	0.247	14.39	0.748
Subsequent milkings	9.6	6.70	0.643	5.29	0.508	4.61	0.443	16.60	1.594
	6.7	6.20	0.415	5.15	0.345	4.71	0.316	16.06	1.076
	7.4	6.95	0.514	5.23	0.387	4.78	0.354	16.96	1.255
	7.0	6.15	0.431	5.08	0.356	4.85	0.340	16.08	1.126
Average preliminary milkings	6.76	6.07	0.410	5.39	0.364	4.63	0.313	16.09	1.088
Average subsequent milkings	7.70	6.52	0.501	5.20	0.399	4.73	0.363	16.45	1.263

TABLE 3

Effect of an incomplete milking

Cow 278, pure bred Guernsey; age four years one month; calved May 5, 1922; milked two times per day; March 26, p.m. to April 6, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	15.0	5.95	0.893	5.38	0.807	4.14	0.621	15.47	2.321
	15.1	4.60	0.695	5.36	0.809	4.11	0.621	14.07	2.125
	17.6	5.50	0.968	5.19	0.913	4.08	0.718	14.77	2.600
	15.8	4.75	0.751	5.34	0.844	4.01	0.634	14.10	2.228
	16.7	5.25	0.877	5.35	0.893	3.97	0.663	14.57	2.433
	15.0	5.15	0.773	5.46	0.819	4.19	0.629	14.80	2.220
	16.7	5.2	0.868	5.28	0.882	3.85	0.643	14.33	2.393
	15.3	5.0	0.765	5.56	0.851	3.91	0.598	14.47	2.214
Incomplete milking	11.7	3.7	0.433	5.37	0.628	4.07	0.476	13.14	1.537
Subsequent milkings	20.0	5.35	1.070	5.13	1.026	3.95	0.790	14.43	2.886
	14.9	5.65	0.842	5.14	0.766	4.04	0.602	14.83	2.210
	15.1	5.10	0.770	5.08	0.767	4.09	0.618	14.27	2.155
	14.8	5.10	0.755	5.11	0.756	4.13	0.611	14.34	2.122
Average preliminary milkings	15.9	5.18	0.824	5.36	0.852	4.03	0.641	14.57	2.317
Average subsequent milkings	16.2	5.30	0.859	5.12	0.829	4.03	0.653	14.46	2.343

TABLE 4

Effect of an incomplete milking

Cow 277, pure bred Ayrshire; age four years two months; calved March 2, 1923; milked two times per day; March 26, p.m. to April 6, p.m. 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	15.8	5.65	0.893	5.30	0.837	3.70	0.585	14.65	2.315
	15.2	5.10	0.775	5.49	0.834	3.87	0.588	14.46	2.198
	16.5	4.95	0.817	5.31	0.876	3.55	0.586	13.81	2.279
	15.8	5.40	0.853	5.42	0.856	3.67	0.580	14.49	2.289
	15.6	4.60	0.718	5.28	0.824	3.52	0.549	13.40	2.090
	16.2	5.10	0.826	5.38	0.872	3.65	0.591	14.13	2.289
	16.2	4.50	0.729	5.30	0.859	3.57	0.578	13.37	2.166
	16.6	5.25	0.872	5.37	0.891	3.52	0.584	14.14	2.347
Incomplete milking	11.9	2.95	0.351	5.38	0.640	3.80	0.452	12.13	1.443
Subsequent milkings	19.8	5.75	1.139	5.43	1.075	3.56	0.705	14.74	2.919
	13.9	4.15	0.577	5.42	0.753	3.74	0.520	13.31	1.850
	14.7	5.35	0.786	5.59	0.822	3.70	0.544	14.64	2.152
	15.8	5.10	0.806	5.22	0.825	3.62	0.572	13.94	2.203
Average preliminary milkings	15.99	5.07	0.810	5.36	0.856	3.63	0.580	14.05	2.246
Average subsequent milkings	16.05	5.15	0.827	5.41	0.869	3.65	0.585	14.21	2.281

TABLE 5

Effect of an incomplete milking

Cow 200, pure bred Holstein; age ten years three months; calved August 27, 1922; milked two times per day; April 24, p.m. to May 4, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	15.2	2.90	0.441	4.65	0.707	3.70	0.562	11.25	1.710
	16.7	3.60	0.601	4.58	0.765	3.71	0.620	11.89	1.986
	14.4	3.45	0.497	4.48	0.645	3.78	0.544	11.71	1.686
	15.4	3.50	0.539	4.50	0.693	3.63	0.559	11.63	1.791
	13.2	3.30	0.436	4.58	0.605	3.46	0.457	11.34	1.497
	14.9	3.45	0.514	4.56	0.679	3.72	0.554	11.73	1.748
	13.8	3.70	0.511	4.56	0.629	3.69	0.509	11.95	1.649
	14.6	3.20	0.467	4.57	0.667	3.74	0.546	11.51	1.680
Incomplete milking	7.3	2.80	0.204	4.51	0.329	3.54	0.258	10.85	0.792
Subsequent milkings	18.3	3.40	0.622	4.29	0.785	3.58	0.665	11.27	2.062
	13.7	4.00	0.548	4.21	0.577	3.61	0.495	11.82	1.619
	15.6	3.30	0.515	4.33	0.675	3.84	0.599	11.47	1.789
	15.8	3.60	0.569	4.48	0.708	3.57	0.564	11.65	1.841
Average preliminary milkings	14.78	3.39	0.501	4.56	0.674	3.68	0.544	11.63	1.718
Average subsequent milkings	15.85	3.56	0.563	4.33	0.686	3.65	0.578	11.53	1.828

TABLE 6

Effect of an incomplete milking

Cow 288, pure bred Holstein; age four years; calved June 27, 1922; milked two times per day; April 24, p.m. to May 4, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	10.3	3.25	0.335	5.12	0.527	4.23	0.436	12.60	1.298
	10.9	3.10	0.338	4.95	0.540	4.15	0.452	12.20	1.330
	9.6	3.45	0.331	5.18	0.497	4.13	0.396	12.76	1.225
	10.5	3.25	0.341	5.01	0.526	4.13	0.434	12.39	1.301
	9.5	3.50	0.333	5.13	0.487	4.31	0.409	12.94	1.229
	9.5	3.70	0.352	5.35	0.508	4.16	0.395	13.21	1.255
	8.1	3.40	0.275	5.05	0.409	4.16	0.337	12.61	1.021
	10.6	3.50	0.371	5.05	0.535	4.20	0.445	12.75	1.352
Incomplete milking	5.2	3.20	0.166	5.15	0.268	4.02	0.209	12.37	0.643
Subsequent milkings	12.9	3.70	0.477	4.98	0.642	4.17	0.538	12.85	1.658
	8.8	4.20	0.370	4.94	0.435	4.16	0.366	13.30	1.170
	10.1	3.45	0.348	5.00	0.505	4.22	0.426	12.67	1.280
	9.5	3.50	0.333	5.14	0.488	4.07	0.387	12.71	1.207
Average preliminary milkings	9.88	3.39	0.334	5.10	0.504	4.18	0.413	12.67	1.251
Average subsequent milkings	10.33	3.70	0.382	5.00	0.517	4.16	0.429	12.87	1.329

TABLE 7

Effect of an incomplete milking

Cow 277, pure bred Ayrshire; age four years three months; calved March 2, 1923; milked two times per day; April 24, p.m. to May 4, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	14.8	3.85	0.570	5.22	0.773	3.97	0.588	13.04	1.930
	14.2	3.70	0.525	5.33	0.757	4.19	0.595	13.22	1.877
	16.0	4.30	0.688	5.02	0.803	3.92	0.627	13.24	2.118
	16.4	4.50	0.738	5.16	0.846	3.86	0.633	13.52	2.217
	13.3	4.70	0.625	5.11	0.680	3.93	0.523	13.74	1.827
	14.3	3.90	0.558	5.12	0.732	3.95	0.565	12.97	1.855
	14.3	4.30	0.615	5.07	0.725	3.87	0.553	13.24	1.893
	14.6	4.70	0.686	5.25	0.767	3.93	0.574	13.88	2.026
Incomplete milking	7.1	2.50	0.178	5.22	0.371	3.89	0.276	11.61	0.824
Subsequent milkings	17.2	5.20	0.894	5.06	0.870	3.67	0.631	13.93	2.396
	13.2	4.85	0.640	5.07	0.669	3.93	0.519	13.85	1.828
	12.8	4.70	0.602	5.34	0.684	3.82	0.489	13.86	1.774
	12.8	5.40	0.691	5.21	0.667	3.66	0.468	14.27	1.827
Average preliminary milkings	14.74	4.25	0.626	5.16	0.760	3.95	0.582	13.35	1.968
Average subsequent milkings	14.00	5.05	0.707	5.16	0.722	3.76	0.527	13.97	1.956

TABLE 8

Effect of an incomplete milking

Cow 254, pure bred Holstein; Age eight years five months; calved October 27, 1922; milked four times per day; May 21, p.m. to May 25, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		lbs.		lbs.		lbs.		lbs.
Preliminary milkings	14.0	2.81	0.393	4.57	0.640	3.70	0.518	11.08	1.551
	13.2	3.10	0.409	4.84	0.639	3.61	0.477	11.55	1.525
	14.2	3.34	0.474	4.96	0.704	3.66	0.520	11.95	1.697
	13.1	3.22	0.422	4.87	0.638	3.50	0.459	11.59	1.518
	14.5	3.03	0.439	4.81	0.697	3.41	0.494	11.24	1.630
	13.5	3.13	0.423	4.78	0.645	3.54	0.478	11.45	1.546
	15.7	3.09	0.485	4.75	0.746	3.57	0.560	11.41	1.791
	13.9	3.27	0.455	4.69	0.652	3.63	0.505	11.59	1.611
Incomplete milking	6.7	2.07	0.139	4.57	0.306	3.66	0.245	10.30	0.690
Subsequent milkings	22.4	2.93	0.656	4.60	1.030	3.66	0.820	11.19	2.507
	15.7	3.12	0.490	4.58	0.719	3.83	0.601	11.53	1.810
	14.4	3.26	0.469	4.65	0.670	3.86	0.556	11.77	1.695
	14.2	2.71	0.385	4.58	0.650	3.53	0.501	10.82	1.536
	15.2	3.00	0.456	4.57	0.695	3.78	0.575	11.35	1.725
	15.8	2.82	0.446	4.57	0.722	3.76	0.594	11.15	1.762
	14.3	3.09	0.442	4.56	0.652	3.84	0.549	11.49	1.643
	14.7	2.79	0.410	4.56	0.670	3.70	0.544	11.05	1.624
Average preliminary milkings	14.01	3.12	0.437	4.78	0.670	3.58	0.501	11.48	1.608
Average subsequent milkings	15.84	2.96	0.469	4.58	0.726	3.74	0.592	11.29	1.788

TABLE 9

Effect of an incomplete milking

Cow 306, pure bred, Jersey; Age six years nine months; calved January 26, 1923 milked four times per day; June 4, p.m. to June 8, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	6.8	4.71	0.320	5.05	0.343	4.58	0.311	14.34	0.975
	6.3	5.50	0.347	5.04	0.318	4.56	0.287	15.10	0.951
	7.1	5.71	0.405	5.07	0.360	4.54	0.322	15.32	1.088
	8.1	4.94	0.400	5.06	0.410	4.74	0.384	14.74	1.194
	6.5	5.29	0.344	5.07	0.330	4.52	0.294	14.88	0.967
	7.5	5.80	0.435	5.05	0.379	4.55	0.341	15.40	1.155
	6.3	4.83	0.304	5.07	0.319	4.63	0.292	14.53	0.916
	6.8	5.25	0.357	5.06	0.344	4.66	0.317	14.97	1.018
Incomplete milking	3.6	3.50	0.126	5.33	0.192	4.77	0.172	13.60	0.490
Subsequent milkings	9.2	5.26	0.484	4.96	0.456	4.51	0.415	14.73	1.355
	5.8	5.43	0.315	5.12	0.297	4.64	0.269	15.19	0.881
	7.2	5.24	0.377	5.20	0.374	4.64	0.334	15.08	1.086
	5.3	5.36	0.284	5.25	0.278	4.62	0.245	15.23	0.807
	8.0	5.01	0.401	5.09	0.407	4.36	0.349	14.46	1.157
	7.2	5.63	0.405	5.06	0.364	4.37	0.315	15.06	1.084
	6.6	5.77	0.381	5.06	0.334	4.53	0.299	15.36	1.014
	7.0	5.55	0.389	5.22	0.365	4.35	0.305	15.12	1.058
Average preliminary milkings	6.93	5.26	0.364	5.06	0.350	4.60	0.318	14.92	1.033
Average subsequent milkings	7.04	5.39	0.379	5.11	0.359	4.50	0.316	15.00	1.055

TABLE 10

Effect of an incomplete milking

Cow 297, pure bred Guernsey; age six years seven months; calved November 20, 1922; milked four times per day; June 11, p.m. to June 15, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
inary milkings.........	8.7	4.26	0.371	5.34	0.465	4.12	0.358	13.72	1.194
	9.1	4.86	0.442	5.37	0.489	4.40	0.400	14.63	1.331
	10.6	5.04	0.534	5.34	0.566	4.25	0.451	14.63	1.551
	9.6	5.23	0.502	5.24	0.503	4.28	0.411	14.75	1.416
	10.1	4.83	0.488	5.33	0.538	4.08	0.412	14.24	1.438
	8.8	4.98	0.438	5.33	0.469	4.32	0.380	14.63	1.287
	11.0	5.27	0.580	5.37	0.591	4.31	0.474	14.95	1.645
	9.3	5.43	0.505	5.28	0.491	4.20	0.391	14.91	1.387
complete milking............	4.6	3.80	0.353	5.30	0.493	4.21	0.392	13.31	1.387
Subsequent milkings.........	13.3	4.43	0.589	5.37	0.714	4.34	0.577	14.14	1.881
	10.0	8.96	0.896	5.28	0.528	3.71	0.371	17.95	1.795
	8.2	5.31	0.434	5.34	0.438	4.38	0.359	15.03	1.232
	8.8	4.86	0.428	5.26	0.463	4.19	0.369	14.31	1.259
	8.7	5.07	0.441	5.31	0.462	4.49	0.391	14.87	1.294
	10.9	5.21	0.568	5.29	0.577	4.27	0.465	14.77	1.610
	8.9	5.47	0.487	5.30	0.472	4.17	0.371	14.94	1.330
	9.8	4.61	0.452	5.32	0.521	4.16	0.408	14.09	1.381
Average preliminary milkings..	9.65	5.00	0.482	5.33	0.514	4.25	0.409	14.57	1.406
Average subsequent milkings...	9.83	5.46	0.537	5.31	0.522	4.21	0.414	14.99	1.472

TABLE 11

Effect of an incomplete milking

Cow 263, pure bred Holstein; age five years six months; calved October 28, 1922; milked four times per day; June 18, p.m. to June 22 p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	17.6	3.39	0.597	4.52	0.796	3.37	0.593	11.28	1.985
	14.8	3.35	0.496	4.71	0.697	3.58	0.530	11.64	1.723
	16.5	2.52	0.416	4.59	0.757	3.39	0.559	10.50	1.733
	16.8	3.09	0.519	4.56	0.766	3.35	0.563	11.00	1.848
	15.6	2.83	0.441	4.59	0.716	3.92	0.612	11.34	1.769
	14.5	3.32	0.481	4.75	0.689	3.50	0.508	11.57	1.678
	17.7	3.33	0.589	4.66	0.825	3.68	0.651	11.67	2.066
	15.4	3.22	0.496	4.54	0.699	3.66	0.564	11.42	1.759
Incomplete milking	7.9	2.19	0.173	4.61	0.364	3.44	0.272	10.24	0.809
Subsequent milkings	20.1	2.86	0.575	4.56	0.917	3.70	0.744	11.12	2.235
	18.0	2.94	0.529	4.47	0.805	3.49	0.628	10.90	1.962
	16.8	3.40	0.571	4.49	0.754	3.54	0.595	11.43	1.920
	15.1	2.60	0.393	4.50	0.680	3.56	0.538	10.66	1.610
	18.6	3.06	0.569	4.62	0.859	3.67	0.683	11.35	2.111
	18.2	2.99	0.544	4.46	0.812	3.49	0.635	10.94	1.991
	16.2	2.98	0.483	4.39	0.711	3.72	0.603	11.09	1.797
	17.7	3.22	0.570	4.49	0.795	3.60	0.637	11.31	2.002
Average preliminary milkings	16.11	3.13	0.504	4.61	0.743	3.55	0.572	11.30	1.820
Average subsequent milkings	17.59	3.01	0.529	4.50	0.791	3.60	0.633	11.11	1.953

TABLE 12

Effect of an incomplete milking

Cow 297, pure bred Guernsey; age six years seven months; calved November 20, 1922; milked four times per day; June 25, p.m. to June 29, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	8.6	4.32	0.372	5.20	0.447	3.97	0.341	13.49	1.160
	7.7	4.21	0.324	5.17	0.398	4.32	0.333	13.70	1.055
	9.0	4.89	0.440	5.27	0.474	4.11	0.370	14.27	1.284
	7.9	4.33	0.342	5.24	0.414	4.22	0.333	13.79	1.089
	10.3	5.09	0.524	5.21	0.537	3.98	0.410	14.28	1.471
	7.0	4.72	0.330	5.40	0.378	4.29	0.300	14.41	1.009
	9.6	4.95	0.475	5.29	0.508	4.03	0.387	14.27	1.370
	8.4	5.49	0.461	5.27	0.443	4.24	0.356	15.00	1.260
Incomplete milking	3.5	3.70	0.130	5.30	0.186	3.90	0.137	12.90	0.452
Subsequent milkings	13.0	4.71	0.612	5.37	0.698	4.31	0.560	14.39	1.871
	9.1	6.02	0.548	5.48	0.497	4.40	0.400	15.90	1.447
	8.1	5.69	0.461	5.35	0.433	4.26	0.345	15.30	1.239
	8.6	4.45	0.383	5.24	0.451	4.25	0.366	13.94	1.199
	8.2	4.97	0.408	5.37	0.440	4.34	0.356	14.68	1.204
	9.6	5.33	0.512	5.36	0.515	4.16	0.399	14.85	1.426
	8.4	4.64	0.390	5.34	0.449	4.24	0.356	14.22	1.194
	9.8	5.07	0.497	5.20	0.510	4.00	0.392	14.27	1.398
Average preliminary milkings	8.56	4.77	0.408	5.25	0.450	4.13	0.353	14.16	1.212
Average subsequent milkings	9.35	5.10	0.476	5.34	0.499	4.24	0.394	14.81	1.384

TABLE 13

Effect of an incomplete milking

Cow 282, pure bred Guernsey; age eight years eleven months; calved February 22, 1923; milked four times per day; July 9, p.m. to July 13, p.m., 1923.

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	11.5	4.01	0.461	5.38	0.619	3.96	0.455	13.35	1.535
	11.2	4.17	0.467	5.47	0.613	4.01	0.449	13.65	1.529
	10.6	4.12	0.437	5.57	0.590	4.10	0.435	13.79	1.462
	11.4	4.39	0.500	5.51	0.628	3.98	0.454	13.88	1.582
	11.2	4.02	0.450	5.50	0.616	3.82	0.428	13.34	1.494
	12.0	4.45	0.534	5.50	0.660	3.86	0.463	13.81	1.657
	11.0	4.05	0.446	5.52	0.607	4.04	0.444	13.61	1.497
	11.5	4.68	0.538	5.50	0.633	4.11	0.473	14.29	1.643
Incomplete milking	5.5	3.35	0.184	5.51	0.303	3.59	0.197	12.45	0.685
Subsequent milkings	15.5	6.67	1.034	5.24	0.812	4.09	0.634	16.00	2.480
	11.6	4.92	0.571	5.37	0.623	3.89	0.451	14.18	1.645
	11.1	4.71	0.523	5.35	0.594	4.03	0.447	14.09	1.564
	11.0	4.26	0.469	5.38	0.592	3.89	0.428	13.53	1.848
	11.0	4.47	0.492	5.43	0.597	3.93	0.432	13.83	1.521
	10.8	4.19	0.453	5.48	0.592	4.03	0.435	13.70	1.480
	10.5	4.50	0.473	5.50	0.578	3.90	0.410	13.90	1.460
	11.3	3.90	0.441	5.38	0.608	3.81	0.431	13.09	1.479
Average preliminary milkings	11.3	4.24	0.479	5.49	0.620	3.98	0.450	13.72	1.549
Average subsequent milkings	11.6	4.80	0.557	5.38	0.624	3.95	0.458	14.13	1.639

TABLE 14

Effect of an incomplete milking

Cow 302, pure bred Holstein; age two years eleven months; calved January 1, 1923; milked four times per day; July 16, p.m. to July 20, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	12.0	2.66	0.319	5.02	0.602	3.79	0.455	11.47	1.376
	11.9	2.86	0.340	5.11	0.608	3.86	0.459	11.83	1.408
	12.9	2.57	0.332	5.13	0.662	3.69	0.476	11.39	1.469
	12.6	2.84	0.358	5.16	0.650	3.69	0.465	11.60	1.473
	11.8	2.58	0.304	5.02	0.592	3.72	0.439	11.32	1.336
	11.8	2.73	0.322	5.23	0.617	3.71	0.438	11.67	1.377
	12.5	2.61	0.326	5.08	0.635	3.59	0.449	11.28	1.410
	11.4	2.90	0.331	5.13	0.585	3.56	0.406	11.59	1.321
Incomplete milking	5.9	2.19	0.129	5.13	0.303	3.72	0.219	11.04	0.651
Subsequent milkings	15.5	2.86	0.443	5.18	0.803	3.75	0.581	11.79	1.827
	12.5	2.55	0.319	5.12	0.640	3.66	0.458	11.33	1.416
	11.3	2.72	0.307	5.14	0.581	3.64	0.411	11.50	1.300
	11.9	2.66	0.317	5.11	0.608	3.50	0.417	11.27	1.341
	12.4	2.76	0.342	5.18	0.642	3.54	0.439	11.48	1.424
	12.4	2.86	0.355	5.24	0.650	3.59	0.445	11.69	1.450
	11.4	2.94	0.335	5.17	0.589	3.67	0.418	11.78	1.343
	12.3	2.80	0.344	5.19	0.638	3.74	0.460	11.73	1.443
Average preliminary milkings	12.11	2.72	0.329	5.11	0.619	3.70	0.448	11.53	1.396
Average subsequent milkings	12.46	2.77	0.345	5.17	0.644	3.64	0.453	11.58	1.443

TABLE 15

Effect of an incomplete milking

Cow 269, pure bred Jersey; age twelve years; calved July 1, 1923; milked four times per day; July 23, p.m. to July 27, p.m., 1923

	MILK YIELD	FAT		LACTOSE		PROTEIN + ASH		TOTAL SOLIDS	
		Per cent	Yield	Per cent	Yield	Per cent	Yield	Per cent	Yield
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
Preliminary milkings	6.3	6.08	0.383	4.72	0.297	3.74	0.236	14.54	0.916
	5.3	5.30	0.286	4.68	0.248	3.53	0.187	13.60	0.721
	5.5	4.40	0.242	4.88	0.268	3.76	0.207	13.04	0.717
	5.5	4.42	0.243	4.86	0.267	3.59	0.197	12.87	0.708
	5.7	4.99	0.284	4.88	0.278	3.41	0.194	13.28	0.757
	5.3	6.47	0.343	4.89	0.259	3.21	0.170	14.57	0.772
	4.9	5.20	0.255	4.87	0.239	3.40	0.167	13.47	0.660
	5.1	5.72	0.292	5.85	0.247	3.44	0.175	14.01	0.715
Incomplete milking	2.7	4.34	0.117	4.86	0.131	3.55	0.096	12.75	0.344
Subsequent milkings	7.1	5.98	0.425	4.76	0.338	3.28	0.233	14.02	0.995
	5.2	5.31	0.276	4.68	0.243	3.46	0.180	13.45	0.699
	5.1	4.52	0.231	4.79	0.244	3.41	0.174	12.72	0.649
	5.1	6.45	0.329	4.69	0.239	3.49	0.178	14.63	0.746
	4.2	5.83	0.245	4.84	0.203	3.24	0.136	13.91	0.584
	5.0	5.46	0.273	5.00	0.250	3.47	0.174	13.93	0.697
	4.4	4.16	0.183	4.94	0.217	3.50	0.154	12.60	0.554
	4.2	5.13	0.215	4.93	0.207	3.62	0.155	13.68	0.575
Average preliminary milkings	5.45	5.34	0.291	4.82	0.263	3.52	0.191	13.68	0.745
Average subsequent milkings	5.04	5.40	0.272	4.82	0.242	3.43	0.173	13.65	0.687

TABLE 16

Number of cows showing a significant average increase or decrease in the percentage fat, lactose, and protein + ash contents and the yields of fat and milk

	MILK YIELD	FAT		LACTOSE	PROTEIN + ASH
		Yield	Per cent	Per cent	Per cent
	lbs.	*lbs.*			
Trial I—4 cows (A):					
Number increased	1	2	2		2
Number decreased				3	
Trial II—3 cows (B):					
Number increased	1	3	3		
Number decreased	1			2	1
Trials III to X—8 cows (C):					
Number increased	3	4	4		2
Number decreased			2	3	1

* A significant average increase or decrease was considered as not less than 0.1 per cent for percentages, 0.7 pounds for yield of milk, and 0.03 pounds for yield of fat.

(A) Cows milked 2 times per day, one-fourth of average milk yield left in udder.
(B) Cows milked 2 times per day, one-half of average milk yield left in udder.
(C) Cows milked 4 times per day, one-half of average milk left in udder.

TABLE 17

Comparison of the grand averages of all the average increases or decreases in the respective percentage fat, lactose, and protein + ash contents and the yields of fat and milk, as determined for each trial

	MILK YIELD	FAT		LACTOSE	PROTEIN + ASH
		Yield	Per cent	Per cent	Per cent
	lbs.	*lbs.*			
Trial 1 (A), tables 1 to 4, inclusive:					
Average increase*	0.43	0.048	0.17	0.05	0.08
Average decrease*	0.46			0.22	
Trial 2 (B), tables 5 to 7, inclusive:					
Average increase*	0.76	0.064	0.40		
Average decrease*	0.74			0.20	0.10
Trials 3 to 10 (C), tables 8 to 15, inclusive:					
Average increase*	0.72	0.041	0.28	0.07	0.10
Average decrease*	0.41	0.019	0.13	0.14	0.05

* These figures represent the grand average of all the increases or decreases, which are due to differences between the averages of the respective percentage contents and yields. The averages of these respective percentage contents and yields being determined from the preliminary and subsequent milkings reported in the tables included in each trial.

(A), (B), (C) See table 16.

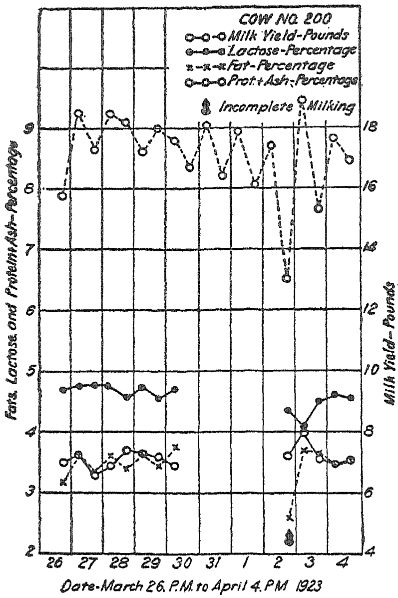


Fig. No. 1

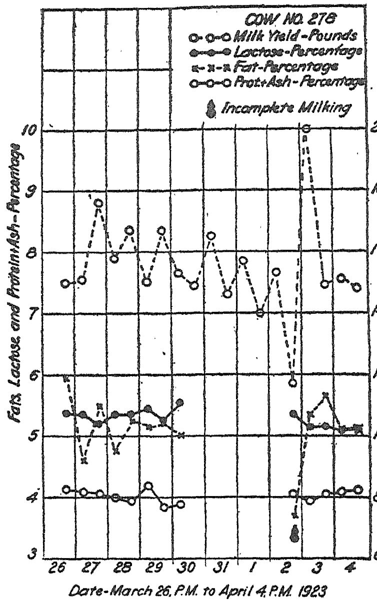
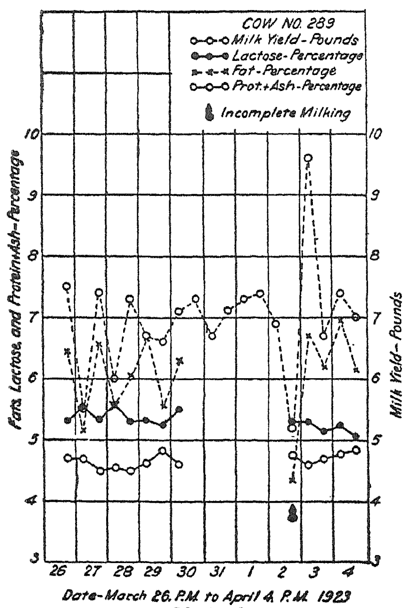


Fig. No. 3



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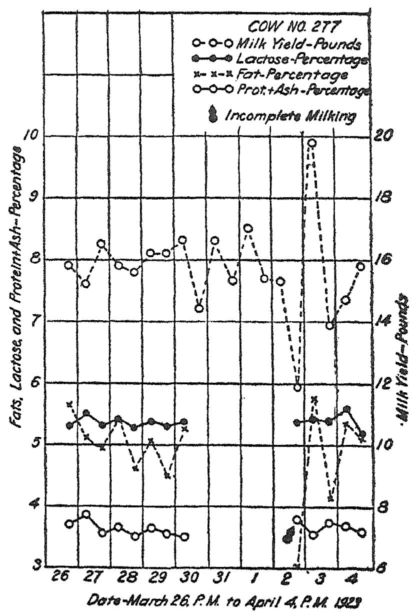

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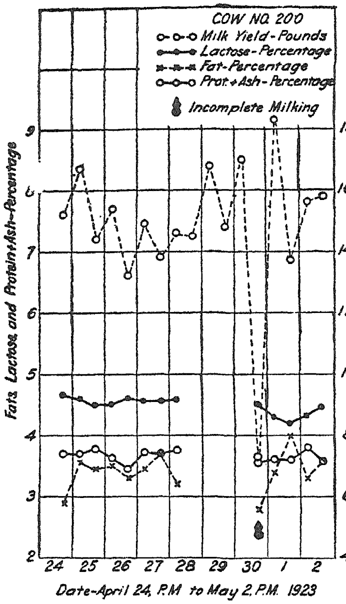


Fig. No. 5

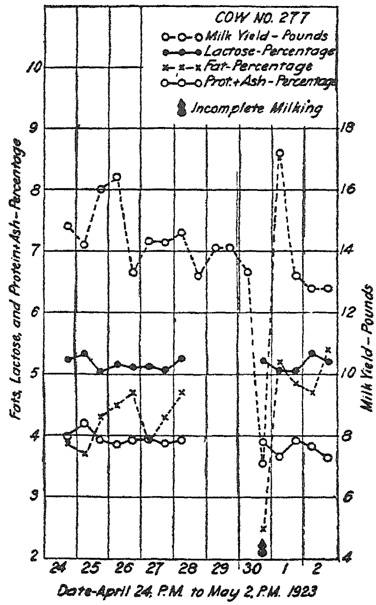
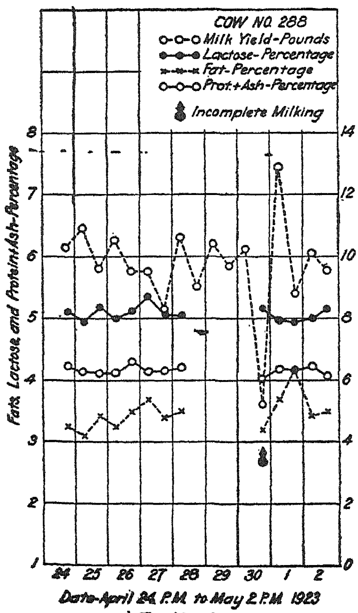


Fig. No. 7



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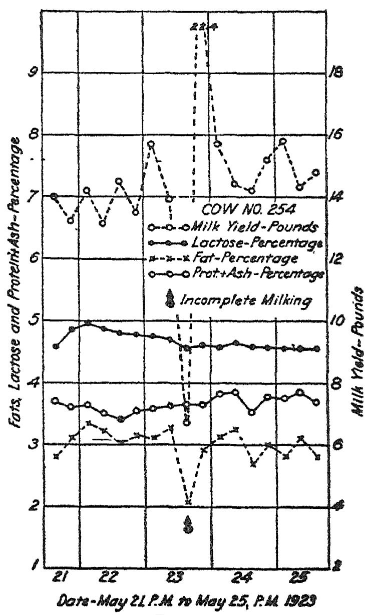


Fig. No. 8

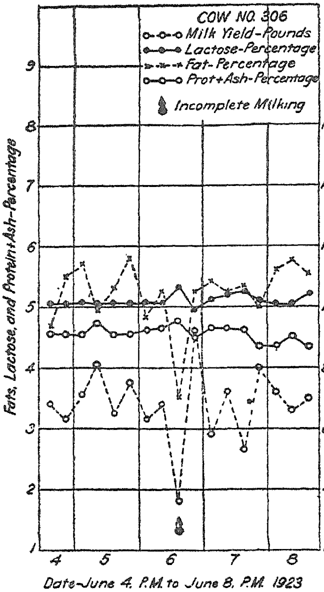


Fig. No. 9

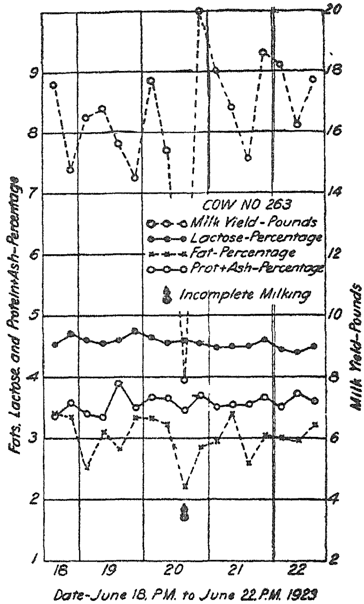


Fig. No. 11

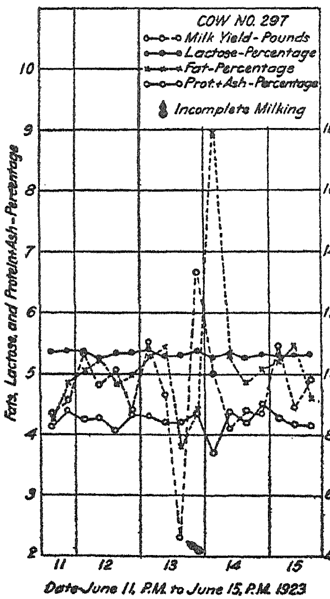


Fig. No. 10

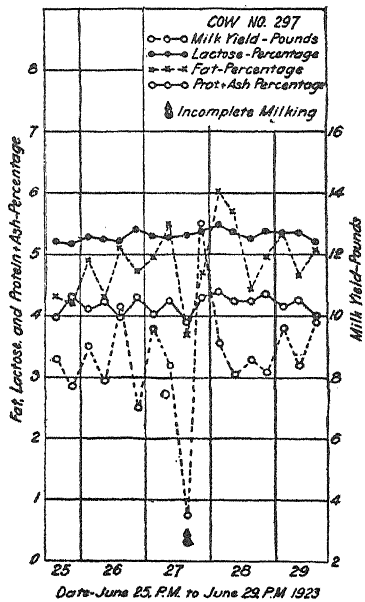
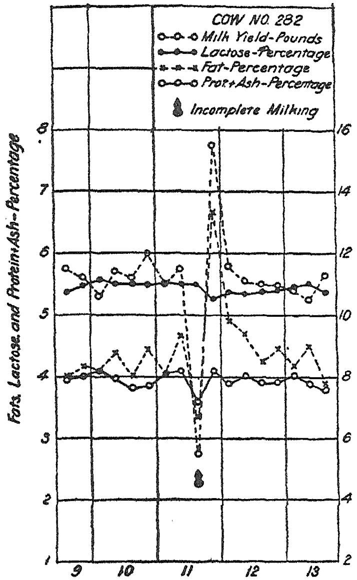
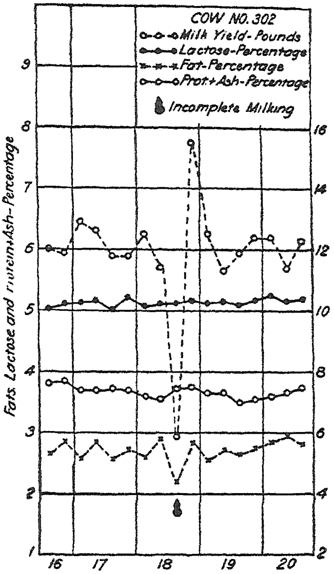


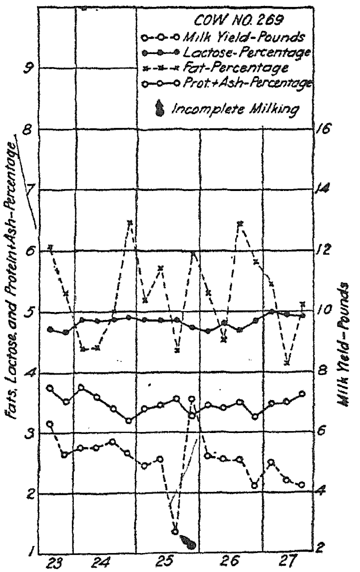
Fig. No. 12



Date-July 9. P.M. to July 13. P.M. 1923
Fig. No. 13



Date-July 16. P.M. to July 20. P.M. 1923
Fig. No. 14



Date-July 23. P.M. to July 27. P.M. 1923
Fig. No. 15

Tables 5 to 7 and figures 5 to 7 include the results of the second trial. The cows were milked twice a day during this trial and one-half of the average milk yield was left in the udder. Of the 3 cows tested, one showed a significant average increase in the yield of milk, and one a significant average decrease in the yield of milk. All three cows showed a significant average increase in both the percentage fat content and yield of fat. Two of the 3 cows showed a significant average decrease in the percentage lactose content, and one a significant average decrease in the percentage protein + ash content.

Tables 8 to 15 and figures 8 to 15 include the results of trials III to X. The cows were milked 4 times a day during these trials and one-half of the average milk yield was left in the udder. Each trial involved the use of one cow, the Guernsey cow 297 was run twice once in trial V and once in trial VII. Of the 8 cows tested, 3 showed a significant average increase in milk yield, 4 a significant average increase in both the percentage fat content and yield of fat, and 2 a significant average decrease in the percentage fat content. Three of the 8 cows showed a significant average decrease in the percentage lactose content and 2 a significant average increase in the percentage protein + ash content. The Guernsey cows 282 and 297 and the Jersey cow 306 were the only cows to show a significant average increase in the percentage fat content. Only one Holstein cow, 254, showed a significant average increase in the yield of fat, this increase being due to a large increase in the milk yield and not in the percentage fat content. Of the 3 cows showing a significant average decrease in the percentage lactose content, 2 were Holsteins and the other a Guernsey. It might be of interest to point out that all cows in trials III to X showing a significant average increase in the percentage fat content were of the high testing breeds.

SUMMARY

The difference between the averages of the respective percentage fat lactose and protein + ash contents and the yields of fat and milk, as determined from the preliminary and subsequent milkings reported for each cow in tables 1 to 15, are in most cases

not very great. There is, however, a general trend in these differences (table 17) which indicates that they are due to the presence of some common disturbing factor, that factor being the leaving of milk within the udder. The differences in the percentage lactose and protein + ash contents are for the most part not as great as those found in the percentage fat content and yields of fat and milk. On the other hand the extent of the variation within the percentage fat content and yields of fat and milk, as measured from one milking to another, figures 1 to 15, is on the average more than twice as great as the equivalent variation within the percentage lactose and protein + ash contents. Hence taking into consideration the degree of variation as measured from one milking to another, it is obvious that comparatively smaller differences in the percentage lactose and protein + ash contents are just as significant as larger differences in the percentage fat content and yields of fat and milk.

Considering the averages of all the increases and decreases in the respective percentage fat, lactose, and protein + ash contents and the yields of fat and milk, as determined for each trial (table 17), it will be found that the average of all the increases and decreases in trial II is greater than the average of all the increases and decreases in trials I or trials III to X. This difference in the averages of all the increases and decreases in the percentage contents and yields as found in the various trials can be attributed to the influence of one of two factors; namely, (1) the amount of milk left in the udder; and, (2) the length of time the milk is left in the udder. In trials I and II the cows were milked twice a day, but in trial 1 one-fourth of the average milk yield was left in the udder, whereas in trial II one-half of the average milk yield was left in the udder. Hence by increasing the amount of milk left in the udder, the effect upon the percentage fat, lactose and protein + ash contents and the yields of fat and milk was increased. In trials III to X the cows were milked 4 times a day and one-half of the average milk yield was left in the udder. In trials III to X the same amount of milk was left in the udder as in trial II, but the milk remained in the udder only 6 hours in trials III to X as compared to twelve hours in trial II. We

find that the average of all the increases and decreases in the various percentage contents and yields in trials III to X is less than the equivelant average in trial II, the decrease being due to the shortening of the length of tine the milk was left in the udder.

The number of cows in each trial showing a significant average increase or decrease in the percentage fat, lactose, and protein + ash contents and the yields of fat and milk (table 16) also manifest the influence of the above mentioned factors in each trial. Trial I included 4 cows and each cow had the opportunity of showing a significant average increase or decrease in the percentage fat, lactose, and protein + ash contents and the yields of fat and milk. If each cow in every trial showed a significant average increase or decrease in every percentage content and yield, there would be 20 significant averages increase or decreases in trial I, 15 significant average increases or decreases in trial II and 40 significant average increases or decreases in trials III to X. Hence the ratio of the actual number of significant average increases or decreases to the total possible number of significant average increases or decreases in each trial will give the amount of disturbance in the various percentage contents and yields due to the leaving of milk within the udder. In trial I we find 10 actual significant average increases or decreases out of a possible 20 significant average increases or decreases, a ratio of 1:1. In trial II we find 10 significant average differences out of a possible 15 significant average differences, a ratio of 2:1. In trails III to X we find 19 actual significant average differences out of a possible 40 significant average differences, a ratio of less than 1:1. Trial II again stands out as showing the most marked disturbance in the various percentage contents and yields due to the amount of milk left in the udder and the length of time it was left in the udder.

CONCLUSIONS

The effect of an incomplete removal of milk from the udder on the quantity and composition of the milk produced during the subsequent two-day period is as follows:

1. The average yield of milk and fat, and the average percentage of fat in the milk tends to increase for the two days following an incomplete milking.

2. The average percentage of lactose in the milk tends to decrease for the two days following an incomplete milking.

3. The average percentage of protein + ash in the milk is changed only very slightly during the two days following an incomplete milking.

4. The degree of change in the composition of the milk produced during the two days following an incomplete milking is influenced by two factors, (a) the amount of milk left in the udder at the incomplete milking, and (b) the length of time the milk is left in the udder, i.e. the length of time elapsing between the incomplete milking and the first succeeding dry milking.

5. The decrease in the percentage of lactose in the milk at any milking during the two days subsequent to an incomplete milking is not sufficient to permit the use of a sugar determination as a detective measure. Such a decrease, however, may be sufficient to justify suspicion.

6. The changes in the composition of the milk produced during the two days following an incomplete milking are not in themselves sufficient to be used as a detective measure without some knowledge of the composition of the milk produced previous to the incomplete milking.

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VITAMINES IN DAIRY PRODUCTS

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Before discussing vitamins it is necessary to know something of the history and nature of vitamins. The name, vitamine, traces back to 1911 when Casimir Funk applied the name to substances that had some curative properties for that dreaded Oriental disease known as beri-beri. This was a disease very common in the Orient where the diet consisted of polished rice. The cause and cure of the disease had never really been understood by the medical men.

Since 1911 many of the best chemists and research men have been working on vitamins and much new information on the subject has been obtained. The scientific and chemical journals have had many articles bearing on the subject, but, as yet, the exact chemical nature of vitamines remains to be determined. At the present time there are three known vitamins necessary for health and growth with two undetermined, one of which prevents rickets, while the other has a marked influence on reproduction. The three vitamins have been named fat solubleA, water soluble B, and water soluble C. The first, as its name implies, accompanies the fat portion of the extracts of cells and tissues; it is the substance necessary for growth and it appears to be the substance that prevents the diseases of rickets and xeropthalmia. It is found most abundantly in milk, eggs, cod liver oil, and in green leaves. The second, or water soluble B, is the substance which protects against neuritis and if absent in the food, beri-beri results. It is found in yeast, in grains and cereals. The third is water soluble C, and is the vitamine that prevents scurvy (1).

Table showing the distribution of the vitamins A, B and C in the common food-stuffs (2)

×××× means very plentiful; ××× abundant; ×× relatively large amount × present in small quantities; 0 absent; (?) not definitely proved.

Foodstuffs	A	B	C
Meats:			
Beef heart	×	×	(?)
Brains	××	×××	×(?)
Codfish	×	×	(?)
Fish roe	×	××	(?)
Herring and salmon	××	××	(?)
Horse meat	×	×	
Kidney	××	××	
Lean meat	×	×	×
Liver	×	×	(?)
Sweetbreads	0	×××	0
Tinned meats	(?)	Very slight	0
Vegetables:			
Beet root	×	×	××
Beet root juice	(?)	×	×××
Cabbage, dried	×××	×××	×
Cabbage, fresh	×××	×××	××××
Carrots	×××	×××	××
Cauliflower	××	×××	××
Celery	(?)	×××	(?)
Chard	×××	××	(?)
Dasheens	×	××	(?)
Lettuce	××	××	××××
Mangels	××	××	(?)
Onions	(?)	×××	×××
Parsnips	××	×××	
Peas (fresh)	×	××	×××
Potatoes	0	×××	××
Potatoes (sweet)	×××	××	(?)
Rutabaga		×××	
Spinach	×××	×××	×××
Cereals:			
Barley	×	×××	(?)
Bread (white)		×(?)	
Bread (whole meal)	×	×××	(?)
Maize, yellow	×	×××	(?)
Maize, white	0	×××	(?)
Oats	×	×××	0
Rice, polished	0	0	0

	A	B	C
Foodstuffs—*Continued*			
Rice, whole grain	×	×××	0
Rye	×	×××	0
Corn embryo		×××	
Malt extract	0	0	0
Wheat bran	0	×	0
Wheat embryo	××	×××	0
Wheat kernel	×	×××	0
Other Seeds:			
Beans, kidney		×××	
Beans, navy		×××	0
Beans, soy	×	×××	0
Cotton seed	××	×××	
Flaxseed	××	×××	
Hempseed	××	×××	0
Millet seed	××	×××	0
Peanuts	×	××	
Peas (dry)	×(?)	××	0
Sunflower seeds	×		
Fruits:			
Apples		××	××
Bananas	(?)	×	××
Grapefruit		×××	×××
Grape juice		×	×
Grapes	0	×	×
Lemons	0	×××	××××
Limes		××	××
Oranges		×××	××××
Pears		××	××
Raisins		×	×
Tomatoes	××	×××	××××
Tomatoes, canned			×××
Oils and Fats:			
Almond oil	0	0	
Beef fat	×	0	0
Butter	××××	0	0
Cocoanut oil	0	0	0
Codliver oil	××××	0	0
Corn oil	××××	0	0
Cotton seed oil	0(?)	0	0
Egg yolk fat	0	0	0
Fish oils	××	0	0
Lard	0	0	0
Oleo, animal	×	0	0

	A	B	C
Foodstuffs—*Continued*			
Oleo, vegetable	0	0	0
Olive oil	0	0	0
Pork fat	0(?)	0	
Tallow	0	0	0
Vegetable oils	0(?)	0	0
Nuts:			
Almonds	×	×××	
Brazil nut		××××	
Chestnut		×××	
Cocoanut	××	×××	
English walnuts		×××	
Filbert		×××	
Hickory	×	×	×
Pine	×	×	×
Dairy products			
Butter	××××	0	0
Cheese	××	×	(?)
Cream	×××	×	(?)
Eggs	××××	××	0
Milk, condensed	××	×	0
Milk powder, skim	×	×××	×(?)
Milk powder, whole	×××	×××	×(?)
Milk, whole	×××	×××	××
Whey	×	×××	×
Miscellaneous			
Alfalfa	×××	×××	(?)
Blood	Varies with sources		
Clover	×××	××××	(?)
Honey		××	0
Malt extract	0	0	0
Meat extract	0	0	0
Timothy	××	×××	0
Yeast brewers	0	××××	0
Yeast cakes	0	××	0
Yeast extract (Vegex)	0	××××	0

THE ANTI BERI-BERI VITAMINE OR WATER SOLUBLE B

The disease beri-beri has been studied for a long time as it has been very prevalent in the Oriental countries. In Japan the disease was prevalent among the sailors whose diet consisted chiefly of polished rice. Professor Eijkman, in 1897, while caring for prisoners on the Island of Java discovered that chickens fed the boiled polished rice developed the disease beri-beri, which disease caused a numbness followed by edema and cramps of the legs. As the disease advances, partial paralysis takes place and edema becomes more general.

After the Philippines had been acquired by the United States, a medical commission was sent to investigate the conditions and study causes of the disease. After their report, which stated that a faulty diet caused beri-beri, due to the large consumption of polished rice, a law was passed prohibiting the use of polished rice in public institutions and the result was a reduction in the number of patients suffering with the disease. They found that the cortex of the rice grain was curative to beri-beri, and therefore must contain the necessary element. However, no definite name was given to the lacking element, nor was any theory set forth as to the exact cause (2).

In 1906, F.G. Hopkins, who has done very good work on vitamins, concluded from his work with experimental animals that something besides the ordinary nutrients was necessary for growth. He concluded the following from the nutritive work he had done with rats: "No animal can live upon a mixture of pure protein, fat and carbohydrate and organic salts. The animal body is adjusted to live either upon plant tissues or other animals and these contain countless substances other than carbohydrates, proteins and fats" (2).

Fraser and Stanton found that an alcohol extract from rice polishings could be made into a concentrated curative for beriberi.

None of the above named men advanced any theory to account for these phenomena and it remained for Casimir Funk, who worked with fractional extracts testing their value on poly-

neuritic birds. He finally obtained a crystalline substance with high curative properties. He called it vitamine, vita, meaning necessary for life, and amine because it contained nitrogen and he believed it to be one of the amino acids.

The Japanese chemist, Suzuki, at the same time as Funk came out with his results, accomplished about the same, but his work had been going on a little longer, thus his work really preceded Funk's. Other workers soon proved Suzuki's and Funk's vitamine crystals to be impure compounds, and it still remains for someone to determine the exact chemical nature of this vitamine. However, the word vitamine still remains as the name of the curative substance. Osborne and Mendel did the first work on dairy products. They were conducting researches in nutrition of a single purified protein supplemented with fat, carbohydrates, and minerals salts, in such quantities and proportions that the required number of calories was always present. They found that no matter how well proportioned the mixture might be in all the known nutrient requirements, the rats failed to grow unless there were added small amounts of milk. In their work they further discovered that the substances in the milk were distributed in two different parts of the milk, namely the butterfat and in the protein and fat free whey. When either of these ingredients were left out of the diet, the rats ceased to grow. No explanation could be found for this phenomena until Funk published his vitamine hypothesis, when it was immediately linked up with nutrition (2).

During this same period many other experimenters had been working along nutritional lines and the year 1911 became significant in that many of the unexplained phenomena in nutrition could be linked up and explained by the Funk vitamine hypothesis. It is from this date on that the greatest strides in nutrition have been taken.

McCollum's work at Wisconsin showed that egg yolk fat contained the same substance as butterfat, and he named this mysterious factor, vitamine fat soluble A.

Water soluble C, or the antiscorbutic vitamine prevents scurvy. The absence of this substance from the diet has been

studied for a long period. In 1400, cabbage soup was known to prevent scurvy when it was shown that 20 men out of 1400 developed the disease because they refused to eat cabbage soup in a prison camp. As early as 1600, English seamen received rations of lemon juice to prevent scurvy. In 1747 Dr. Lind, a surgeon on the British sailing vessel, Salisbury, proved that lemons and oranges would effect a cure for scurvy. In 1775, cabbage and spinach were known as antiscorbutics, but that their curative factor was lost on drying (2).

In 1912, Holst and Frohlich began the study of scurvy which ended in the discovery of a vitamine which was called vitamine water soluble C. Dr. McCollum also worked on scurvy, and in 1918, stated that scurvy was not due to the lack of a vitamine in the diet, but to the absorption of toxins from the intestines, due to protein putrefaction and constipation. His explanation did not agree with other investigators who held to the vitamine hypothesis. He was attacked by many of the investigators in England, and especially by Osborne and Mendel, in America. As time went on, the findings of McCollum were proved erroneous because the injection of orange juice intravenously showed the same curative powers as if ingested. It was also proven that milk contained the factor in question by showing vitamine A and B to be non-antiscorbutic. The vitamine was water soluble like B, but otherwise had different properties.

The proof of the existence of the three vitamines is found in the summary of Prof. F. G. Hopkins researches in physiological chemistry covering fifteen years of his work, which reads as follows: "The absence of one particular vitamine from the food is the whole and sufficient cause of human scurvy, the absence of another forms the main factor in the etiology (cause) of beriberi (general, neuritis), the absence of a third plays at least a large part in the induction of rickets."

But with the opening up of this new field of research calling for entirely new methods, much contradictory literature has been published, due to inaccurate observations, too limited data and the wrong interpretation of results (3).

Up to 1920 it was thought that rickets was caused by deficiency in vitamine A, but since then it has been studied more thoroughly. Dr. Hess has suggested tht there is another vitamine that prevents rickets.

C. H. Hunt has more recently studied this last named deficiency disease. He worked with cod liver oil and butter fat and has observed that if cod-liver oil is administered, rickets will not develop in spite of the fact that the diet is low in calcium mineral. Evidently there is something in cod liver oil that prevents rickets under unfavorable conditions. This substance has been provisionally called vitamine D. Butter does not seem to have this anti-rickets vitamine present to any great extent but if the diet contains plenty of mineral matter, then butter will serve the purpose as well as cod liver oil.

He further observed that rays of light that come direct from the sun have activating energy, but not after passing through glass. These direct rays prevent and cure rickets (4).

The latest research experiments have been conducted to discover the reason for sterility in rats. It was observed that rats could be reared and grow normally but can not reproduce on a so called basic ration consisting of 18 per cent casein, 54 per cent cornstarch, 15 per cent lard, 9 per cent milkfat, and 4 per cent salts to which daily doses of 0.4 to 0.5 gram of dried whole yeast is added. The rats may exhibit normal oestrus and ovulation and conceive. The placentae are abnormal and the products of conceptions are invariably resorbed. Ordinarily the natural foodstuffs contain a substance, *X*, which prevents such a sterility or cures it if it has been established. By feeding green leaves, fresh meat or cereals, fertility will be restored.

In some cases the favorable results were obtained when the latter foods were added to the basic rations after ovulation and fertilization had occurred "so that presumptive evidence is secured of normal germ cells but defective uterine function as the specific cause of the disease. Yet the occurrence of the disease in males shows that in this sex the germ cells are diseased and this possibly also is the case in females."

Here again this substance, X, was found to be present in milk fat though in very small amounts, and to restore fertility the diet must be made up of 24 per cent butterfat. Fertility may be restored by increasing the amount of unextracted commercial casein.

This new factor is distinct from "vitamine A" since it was found to be very low in a certain lot of cod-liver oil of proved high "vitamine A" content, and, furthermore the dietary placental disease does not occur when "A" quota is lower than in our basic ration, providing X be present. The water-soluble vitamine "B" cannot be concerned since when vitamine "B" is increased by high yeast dosage or additional daily feeding of 10 cc. of fresh milk, the disease is not affected. Vitamine "C" seems definitely eliminated by the ineffectual outcome of daily fresh orange juice dosage and by the effectual results of cereals notoriously low in or devoid of "C." The new dietary X can be extracted by alcohol and ether from the curative foods. Studies are being conducted on the characteristics of this factor indispensable for reproduction and on its further distribution in natural foods (5).

From the varied results obtained by many different investigators it would seem that other factors enter in besides experimental errors.

The vitamins were from several substances and collected at different seasons of the year. Recently it has been conclusively proved that the season of the year has had a marked influence on the vitamine content of milk.

To the last named factor many of the conflicting results may be traced. As early as 1916, McCollum, Simmonds, and Fitz had reached the conclusion that vitamins A and B passed in to the the milk only as they were present in the diet of the cow. In 1918, Steenboch, Boutwell, and Kent observed that butterfat varied, and assumed that the ration of the cow might be faulty. Each year has brought out new facts throwing more light on the subject so that at the present time it has been conclusively proved that the A, B and C vitamine content of the food fed to the cow determines the A, B and C vitamine content of the milk (8). C. H. Hunt concluded from experiments conducted at Ohio Agricultural Experiment Station that the milk of cows on green pasture is richer in vitamine C than milk from cows on

dry feed (4). Kennedy and Dutcher showed in their work that vitamins A and B were dependent upon the vitamine content of the ration but showed that it was possible to produce a vitamine-rich milk in the winter by the proper choice of feeds (8).

It has been found that vitamine A is closely associated with carotinoids or the yellow pigment found in plants. It has been proven that yellow corn contains more of the vitamine A than the white corn. However, this should not be taken to mean that a highly yellow colored food is rich in vitamine A, but in some cases it may be taken as an indication as has been shown for yellow corn (4).

Other factors that affect the vitamine content are preservatives, alkalies and heat.

Sodium bicarbonate, or baking soda will destroy vitamine B, but when it is combined with buttermilk or tartaric acid the alkali is neutralized and the vitamins are protected (2).

Vitamine A is gradually lost by oxidation and for that reason butter which is a very good source of vitamine A, gradually loses its vitamine when exposed to oxidation and the same holds true of cod liver oil in the process of refining (2).

The greatest loss in vitamins takes place through heat. Drying most foods destroys the C vitamins and boiling for a long period tends to destroy all of vitamine C. Most authors agree that vitamine C is destroyed in milk by pasteurizing at 145°F. for 30 minutes. However, this still remains to be satisfactorily proved.

Feeding experiments have been carried on to determine how the fat soluble A vitamine and water soluble B are affected by heating. The C vitamine, or the antiscorbutic vitamine, is not necessary for growth in mice as it can be synthesized by them. Milk was autoclaved at 10 and 15 pounds pressure for fifteen minutes and fed to growing mice which had been fed for a period of about fifteen days on a diet devoid of vitamins, but complete in respect to protein, carbohydrates, fats and minerals.

The conclusions of the experiments were that the fat soluble A and the water soluble B of milk autoclaved at 10 and 15 pounds pressure for 15 minutes were not affected to any appreciable extent when fed as a source of vitamins (6).

VITAMINS IN CONDENSED AND DRIED MILKS

Hess and Hume state that sweetened condensed milk retains practically all of its vitamine C, and Daniels and Laughlin show that sweetened evaporated or sweetened condensed milk had sufficient supply of A and B vitamins to support normal growth (7).

Cornelia Kennedy concluded from experiments with Spray and Drum process of drying milk that Vitamine B was not affected by either process but "that the vitamine A content of drum dried milk more nearly approximates the original milk in this respect than does milk dried by the Spray process; and that in both kinds of dried milk a change in the vitamine content may be expected which corresponds to changes found in fresh milk, due to seasonal changes in the feed of the cow." She further concluded that milk powders vary in their content of vitamine C according to the process used in preparation. The period of exposure to heat and oxidation is less during the drum drying process and the result is that less of the vitamine C is destroyed. It was found that milk so processed still contains enough of its antiscorbutic properties to protect babies and experimental animals from scurvy (7).

There are still many undetermined factors that influence vitamins which will have to be studied more carefully.

One of the more important factors that has been worked on and is still under experimentation is the effect of pasteurization on vitamine C. Some authors maintain that it is destroyed while others have concluded that it has only been partially destroyed.

For the chemist it still remains to isolate and determine the chemical nature of vitamins. At the present time all that is known about vitamins is that their presence is essential to normal growth and health.

One thing has been definitely proved, that milk does contain three important vitamins though varying in amount due to variations in vitamine content of the rations fed to the cows. Butter is one of the best sources of vitamine A; cream contains both A and B; cheese A and B; powdered milk contains both A and B, also C although not in very large amounts.

From the above facts, milk and dairy products should be used more extensively in the diet to furnish not only the nutrients but the vitamins without which, proper growth and health will not be obtained.

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A more complete bibliography on vitamins is found in Mathews' Physiological Chemistry, p. 853–857, and in lesson 10 of "Ten Little Lessons on Vitamins" by Eugene Christian.

REVIEW OF FOREIGN DAIRY LITERATURE

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Van Der Burg, B., L.i., Laboratory of Dairy Manufacturing and Dairy Science of the College of Agriculture, Wageningen, Holland. Determination of the Per Cent of Salt in Cheese. As published in the Journal of Microbiology and Hygiene of Comparative Tropical Medicine of Parasitic and Infectious Diseases, vol. ix, no. 3.

In this paper the author calls attention to the fact that salt plays a very important rôle in cheese. He explains, that salt exerts not only a direct influence on the flavor of cheese, but that it also indirectly affects it, by producing a preferential media for certain microörganisms. The microörganisms occurring in freshly made cheese vary as to their sensitiveness to a certain per cent brine solution. By this means, cheese ripening is controlled through the per cent of salt in the moisture of the cheese or in other words the concentration of the salt brine.

In the third place, according to the author, salt also exerts an influence on the body and texture of the curd, as the readiness with which the paracasein absorbs and retains moisture, besides depending on the acidity, is also dependent, as shown by Van Dam, on the concentration of the salt. In view of these important influences of salt on flavor, ripening and physical properties of the cheese, it seemed to the author that there is need for a simple and practical determination of the per cent of salt to replace or supplement the present rather complicated and expensive test.

With this in mind and at the request of one of the large Associations of Cheese Factories, he proposes the following test for salt in cheese:

Sample of cheese is taken in the usual manner and ground fine in a small meat grinder.

After thoroughly mixing, 4 grams of the grated cheese is placed into a 100-cc. measuring flask. About 50 to 60 cc. of warm water and 10 cc. of approximately 0.1 N alkali is added. The mixture is shaken at intervals until the cheese is completely dissolved. It is then allowed to cool to room temperature, when 10 cc. of nitric acid (6 N specific gravity 1.2) is added. Water is then added up to the mark. After thorough mixing, it is filtered through a dry filter; 50 cc. of the filtrate measured

out, to which is added 15 cc. of 0.1 N silver nitrate solution. The excess silver is (without filtering off the formed silver chloride) retitrated with 0.1 N sulfo cyanate of ammonium (NH_4 CNS) using 1 cc. of saturated solution of ferric ammonium sulphate as an indicator. The number of cubic centimeters of silver nitrate solution used minus the amount required for a blank trial, multiplied with 0.2925 gives the per cent of salt in cheese. The correction for the blank determination cannot be

TABLE 1

Comparative results of the modified V. D. Burg test and the ash determination for salt in cheese.

SAMPLE NUMBER	METHOD (V. D. BURG)	ASH DETERMINATION	SAMPLE NUMBER	METHOD (V. D. BURG)	ASH DETERMINATION
1	3.05	2.99	4	1.75	1.81
	3.05		5	2.05	2.12
	3.10		6	2.15	2.14
	3.05		7	1.70	1.78
	3.05		8	2.0	2.0
	3.10		9	1.85	1.96
			10	1.75	1.75
2	2.95	2.95	11	1.60	1.69
	2.95	3.01	12	1.75	1.79
	2.95		13	1.40	1.39
	2.95		14	1.85	1.96
	2.95		15	1.95	1.96
			16	1.90	1.94
3	3.25	3.23	17	1.75	1.76
	3.25	3.23	18	1.90	1.92
	3.15	3.22	19	1.85	1.84
	3.15	3.21			
	3.15	3.23			
	3.15	3.21			
	3.15	3.21			
	3.15	3.21			
	3.15	3.21			
	3.15	3.22			

neglected because the alkali used may contain chlorine. To simplify the calculation one can weigh out 3.9 grams instead of 4 grams. In this case the per cent of salt in the cheese is arrived at by multiplying the cubic centimeters of silver nitrate solution with 0.3.

The author has compared the above test with regular ash determination and in table 1, reports the results of the comparison.

The conclusions of the author are that the comparative results as shown in table 1, indicate that the salt content is determined with sufficient accuracy by use of the much simpler titration method.

Hekma, E., Director of the Division of Physiology. Is Fibrin a Physiological Constituent of Normal Milk? 1922 Annual Report of the Agricultural Experiment Station, Hoorn, Netherlands.

In the course of the experiments regarding the process of milk curdling it appeared that under the influence of rennet and acids, delicate threads occur in milk, which participate in the micro structural formation of the coagulation and are very similar to fibrin threads. As a result of this observation the probability of the occurrence of fibrin in normal milk was subjected to an exhaustive test. Consideration was given to the various forms in which fibrin may occur especially the dissolved, the optically negative and the filament state.

In order to test the milk for the presence of fibrin, blood serum and a saturated solution of table salt was used as one test, and the dark field analysis according to the fibrin coloring test of Weigert as still another test.

Fibrin was not found to occur in normal milk, neither in the dissolved nor in the filament state. Consequently It is not permissible to consider the previously mentioned threads, occurring during the process of curdling, as fibrin. Neither can fibrin participate in the creaming process of milk; even although in the course of the experiment the facts showed that the creaming process may be materially favored by blood serum.

Moreover the fact becomes established that the fibrin cannot be looked on as physiological constituent of milk, henceforth any milk in which fibrin is found should be considered as abnormal, even although this abnormality not necessarily need be considered synonym with unfitness.

Nevertheless such milk should at the same time be subjected in other respects to a thorough test for fitness.

AMERICAN DAIRY SCIENCE ASSOCIATION

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idea being to have them calve after the 365-day test is finished. The duration of time the calf is carried during the test period varies from zero days to nine months. The cow's records for each six months of age have been sorted for 365-day milk yield and the duration of time the calf was carried. The milk yields range from 4000 to 24,000 pounds and are divided into classes of a thousand pounds. The duration of time the calf is carried varies from zero months to nine months and is divided into monthly classes. The correlation coefficients for the two variables, milk yield and butter-fat percentage with the duration of pregnancy are given in table 1.

The correlation coefficients of table 1 to some degree bear out the general impression that the fetus exerts a physiological drain on the mother and that this drain is expressed in a reduction in milk yield. The percentage composition of the milk solids as indicated by the butter-fat is not influenced by the presence of the fetus.

The influence of the length of time the calf is carried is least for the young and old cows and greatest as the cows reach the five-year old mark. There are other extraneous complicating factors entering into this relation. From the manner of conducting the advanced registry test it seems entirely probable that the owner will favor in every way possible the cow that he thinks is going to make a large record. Two things will influence his decision; the previous yearly records of the cow in question and the monthly record of the cow commencing this test. If this monthly record is high it would naturally follow that the dairyman believing in the influence of carrying the calf in tending to reduce the milk yield, would not breed his cow with probable high production so early as he would breed the cows which were going to make a relatively lower record. Study of the records show that such a practice is actually followed. The first four months of lactation are studied for the ages three years to three years six months and three years six months to four years. These records are chosen as they closely represent the average in the relation of the intrauterine developments of the fetus to milk yield. As the milk yields are recorded in calendar months, the

INTRAUTERINE DEVELOPMENT OF THE BOVINE FETUS IN RELATION TO MILK YIELD IN GUERNSEY CATTLE[1]

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Experienced physicians and others who come in contact with obstetrical work in man or animals frequently hold the opinion that the development of the fetus is a serious drain on the animal organism. If this animal happens to be in milk production this supposed drain is said to produce a marked depression in the animal's milk yield. In view of the wide credence placed in this hypothesis it is surprising to find that the experimental evidence presented in its support is so slight. The American Guernsey Cattle Club in volume 31 of their advanced registry furnished data on the milk yields and length of time the calf is carried by the cows during the 365-day lactation in which the cows are making their record. These data are analyzed in the following paper to determine the validity of the hypothesis indicated above.

CORRELATION BETWEEN THE MILK YIELD AND DURATION OF PREGNANCY AND BETWEEN THE BUTTER-FAT PERCENTAGE AND DURATION OF PREGNANCY

The data are divided into periods of six months according to the age of the cow thus eliminating any marked influence of the cow's age on her milk yield. Cows on advanced registry tests are generally bred in or after the fourth month of lactation, the

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 156. The Rockefeller Institute for Medical Research has furnished material aid to these animal husbandry investigations in the form of a grant for increased maintenance of the work. This paper presents one phase of these researches as conducted by the Biological Laboratory of the Maine Agricultural Experiment Station.

first month's record is anything from one to thirty-one days in length. As such a record is worth very little for our problem it is omitted. The correlation coefficients for the relation of the

TABLE 1

Correlation coefficients for milk yield with duration of pregnancy within the 365-day lactation (column 2) and butter-fat percentage with duration of pregnancy within the 365-day lactation (column 3)

AGE GROUPS OF COWS	MILK YIELD	BUTTER-FAT PERCENTAGE
1:6–2:0	−0.021 ± 0.059	−0.011 ± 0.059
2:0–2:6	−0.127 ± 0.018	−0.013 ± 0.018
2:6–3:0	−0.199 ± 0 024	0.089 ± 0.024
3:0–3:6	−0.175 ± 0.027	0.040 ± 0.028
3:6–4:0	−0.235 ± 0.029	0.015 ± 0.031
4:0–5:0	−0.275 ± 0.022	−0.022 ± 0.024
5:0–6:0	−0.228 ± 0.039	0.071 ± 0.030
6:0–8:0	−0.174 ± 0.024	−0.040 ± 0.025
8:0–10:0	−0.180 ± 0.036	−0.029 ± 0.037
10:0–15:6	−0.111 ± 0.057	0.041 ± 0.058
Average...............	−0.173	0.014

TABLE 2

Correlation coefficients for the monthly milk yield with time of interuterine development (column 2) and monthly milk yield with yearly milk yield (column 3)

MONTH OF LACTATION	CORRELATION COEFFICIENT	
	Monthly milk yield and duration of interuterine development	Monthly milk yield and 365-day milk yield
Three years to three years six months		
Second..................................	−0.103 ± 0.028	0.757 ± 0.012
Third..................................	−0.136 ± 0.028	0.807 ± 0.010
Fourth..................................	−0.146 ± 0.028	0.834 ± 0.009
Three years six months to four years		
Second..................................	−0.152 ± 0.031	0.713 ± 0.016
Third..................................	−0.170 ± 0.030	0.784 ± 0.012
Fourth..................................	−0.187 ± 0.030	0.812 ± 0.011

monthly milk yields and the duration of time the calf was carried in the 365-day lactation period and the correlation coefficients for the monthly milk yields and 365-day milk yields are given in table 2.

The second column of table 2, shows that the cows selected by the dairymen to carry the calf nine months are slightly less in their milk yield than are the cows selected to carry the calf one month or not at all. This fact is in accord with the supposition expressed above. To remove the influence of this selection on the relation of milk yield to duration of intrauterine development it is necessary to resort to the partial correlation method. In other words we desire to measure the influence of pregnancy on yearly milk yield for a constant milk yield in the second month of lactation—third month of lactation—fourth month of lactation. The third column of table 2 gives the necessary data.

TABLE 3

Correlation between yearly milk yields and duration of time the calf is carried for a constant monthly milk yield

MONTH OF CONSTANT MILK YIELD	PARTIAL CORRELATION COEFFICIENT	CORRELATION COEFFICIENT ÷ PROBABLE ERROR
Three years to three years six months		
Second	-0.151 ± 0.028	5.4
Third	-0.112 ± 0.028	4.0
Fourth	-0.099 ± 0.028	3.5
Three years six months to four years		
Second	-0.183 ± 0.031	5.9
Third	-0.167 ± 0.031	5.4
Fourth	-0.145 ± 0.031	4.7

It will be noted that the correlation coefficient between the production of one month and the production of a subsequent yearly record is high. Such being the case it is clear that the selection of cows to remain barren on the basis of their monthly record being high will automatically increase any correlation that may exist between the yearly milk yield and the duration of time that the cow carries her calf. This disturbing influence can be removed, made constant, through the use of partial correlation coefficients. The correlation coefficients between yearly milk yields and duration of time the calf is carried with the monthly milk yield constant are given in table 3.

Table 3 shows that even when the effect of the selection based on the monthly milk yields is accounted for there remains a significant correlation between the length of time the calf is carried and the milk yield of the mother. It will be noted that the relation becomes less as the months of lactation advance. Such a result shows that the dairyman places more dependence on the third month's production than he does on the second as an indicator of the probable yield of the cow. Still more emphasis is placed on the fourth month than is placed on the third. Such being the case it appears more reasonable to determine the effect of carrying the calf on the milk yield of the fourth month's record as this month will remove most of the effect of the dairyman's conscious selection. From the known relations of the partial correlation coefficients it is possible to form the necessary equations. These are given below.

EQUATIONS TO PREDICT YEARLY MILK YIELD FROM MONTHLY MILK YIELD AND DURATION OF PREGNANCY

Three years to three years six months old.

Yearly milk yield = 2210–87.98 months calf carried +7.902 milk yield fourth month (1)

Three years six months to four years old.

Yearly milk yield = 2717–69.83 months calf carried +7.683 milk yield sixth month (2)

Solving these equations for the effect of carrying the calf on the milk yield of the mother we find that a cow carrying her calf nine months vs. one which is barren reduces her milk flow by the following amounts depending on the equation used: (1) 342 pounds of milk; (2) 628 pounds of milk.

If this value is converted into therms (therm is taken to be 1000 large calories as defined by Henry in "Feeds and Feeding" and not therm equaling small calorie of the Condensed Chemical Dictionary) of energy it is found that the bovine fetus during gestation uses up approximately 125 to 200 therms of energy considering that a pound of milk represents 337 calories (1).

These figures for the requirements of the bovine fetus are three to four times as large as those of Eckles (2). The difference in

methods of solving the problem seems to be responsible for the difference in results. Thus Eckles calculates the requirements of the fetus on the basis of its chemical composition in relation to the energy required to produce milk of similar composition. In the writer's view this should lead to a value which is too low as the living fetus would constantly increase its energy waste as its volume increased.

The results of Brody, Ragsdale and Turner (3) tend to confirm these conclusions although there is some question as to the ages of the cows dealt with in their work and whether or not any attempt was made to correct the results for any of the extraneous variables which might influence them.

In their essential conclusions the results of the three methods are not very far different. The average milk yield of three and one-half year old Guernsey advanced registry cows is 9100 pounds. On the basis of 500 pounds of milk as the requirement of the fetus the fetus requires for nutriment about 5 per cent of the average milk yield at this age. The range of milk yield for the same aged cows is from 4000 to 18,000 pounds or 14,000 pounds. The fetus requires for its development only 3 per cent of this range. The amount of energy required by the fetus is consequently but a very small part of the energy required for milk yield. Compared with the energy the cow is able to convert, the drain of the fetus on the cow is small.

Incidentally it is of interest to note that the severity of the effect of carrying the calf on the milk yield of the mother seems to increase slightly as the cow nears the time of parturition. The differences, for the cows aged three years and three years six months, while not very large, are probably significant.

SUMMARY

This paper presents a study of advanced registry Guernsey records to determine the effect of carrying the calf on the milk yield of the mother during that pregnancy. The data show that the carrying of the calf is slight but significant drain on the mother's milk producing capacity amounting to from 400 to 600

pounds of milk with an energy value of about 125 to 200 therms. The carrying of the fetus has no influence on the butter-fat percentage.

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IV. SODIUM HYPOCHLORITE

THE PHENOL COEFFICIENT AND RELATIVE DISINFECTING POWER OF SODIUM HYPOCHLORITE[1]

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Authentic data on the relative disinfecting action of sodium hypochlorite is very sparse. Pusch (1) reported the results of some generalized experiments in 1908. While his work dealt in the use of electrolytic bleach liquors of variable content of alkalinity, he expresses his data in the concentration of chlorine per liter of solution, and thus establishes definite points for check measurements. It is noteworthy that this data is of little value to us in our present attack of the problem, because Pusch dealt primarily with organic infested media, such as sewage, blood clots, and profuse cultures of a few bacteria. Sayer and Patty (2) record the results of some tests with sodium hypochlorite wherein infected objects (soda fountain utensils) are immersed for certain periods, and the sterilizing action noted. Dakin and Dunham (3) have given some empirical data of similar value obtained from the use of certain commercial, chlorine derived products.

The more refined methods of examining disinfectants[2] as suggested in the Rideal-Walker, Lancet Commission of Disinfectants,

[1] This paper was read at the annual meeting of the Society of American Bacteriologists, December, 1922, at Detroit, Mich.

[2] There is nothing in the terminology or method of the "phenol coefficient" which implies any specific action of the disinfectant when applied in practice. If we are careful to avoid interpreting the results of a phenol coefficient determination as being directly commensurate with the disinfectant's power in all kinds of products under any set of conditions, without any regard for the chemical structure and properties of the disinfectant or the disinfectant medium, we can still look upon the phenol coefficient as a useful biological meter. It was devised to measure the relative distinctive action of chemicals upon bacterial growth (reproduction) under a given set of conditions. The most insiduous opponent of this test cannot do other than agree in general with the relative coefficients of phenol vs. mercuric chloride, phenol vs. thymol, phenol vs. boric acid. These bear out in practice the general order of the determined coefficients.

or the United States Hygienic Laboratory Method, have not been applied in those mentioned above. No regard for the reaction (pH) of the disinfecting solution, or its chance effect upon the disinfecting power, is apparent in the work.

SCOPE OF THE PRESENT STUDY

First: We have attempted to employ the Hygienic Laboratory Method (4) with only slight modification in our study of sodium hypochlorite disinfection. The modification is as follows:

a. Substitution of 0.3 per cent $Na_2HPO_4 \cdot 2H_2O$ and 0.2 per cent NaCl in lieu of 0.5 per cent NaCl in the culture medium to buffer the reaction between 6.9 and 7.1 pH.

b. The expression of all master dilutions and final dilutions of disinfectants in percentage strength.

The use of typhoid bacilli as a criterion of disinfecting power is open to criticism, since the vitality of bacterial cells, in general, is not characterized by any particular species. But for the sake of relative data alone we may as well choose the typhoid bacilli for the purpose of comparing one disinfectant with another, under like conditions. We have therefore determined the phenol coefficient, within reasonable limits, of sodium hypochlorite using a virile culture of typhoid (Hopkin's) organisms. The original cultures used in this investigation were furnished through the courtesies of the Digestive Ferments Company, of Detroit, and the Army Medical Museum, of Washington.

Second: Sodium hypochlorite, in dilute and concentrated solutions, acts in a dual rôle towards organic matter. It is (*a*) a strong chlorinating substance, and (*b*) a strong oxidizing substance. The former is exhibited in solutions of low pH (below pH 8.0), and the latter at higher pH (above pH 9.0). In any event its solutions are distinctly on the side of a very strong oxidizing potential. In this respect sodium hypochlorite differs markedly from most common disinfectants. We would postulate a different type of disinfectant action from it upon bacterial cells. Mercuric chloride combines with and coagulates the protein of the protoplasm. The phenols are supposed to act toxically to the cellular functions, but probably not as a combining agent

in the common view. Chloroform and kindred substances probably anesthetize, whatever this may signify. Formaldehyde actually combines with the protein and like material of the cell. Hydrogen peroxide disinfects by furnishing an atmosphere of oxygen in the vicinity of the normal reducing centers of the cells, and thereby opposes their functions.

Sodium hypochlorite may act in the dual rôle simultaneously upon the living cell. The bound chlorine may actually chlorinate certain compounds within the cell. Or the "available chlorine" (oxygen) may act in the capacity of hydrogen peroxide. Now it is conceivable that bacterial cells of certain species will tolerate more chlorine or oxygen than others. Gillespie (5) has shown that bacteria which liberate free hydrogen, and some which do not, maintain a very high reduction potential. Measurements by Clark have confirmed the finding. Naturally coli bacilli, which readily evolve hydrogen in carbohydrate media, should establish a very strong reducing medium, both within and without the cell. We would expect this bacterium to consume more of a disinfecting agent like sodium hypochlorite or hydrogen peroxide than an organism of the prodigiosus family. In commencing the study this factor was kept in the foreground, and the phenol coefficient was determined within rather wide limits upon a number of organisms: coli (Neapolitanum), typhoid (Hopkins), proteus vulgaris, alkaligines, gonococcus, anthracis, and tubercle bacilli.

Third: The relative disinfecting power of NaOCl and mercuric chloride on B. coli was determined by the same method used in the phenol coefficient study. The results are expressed as the mercuric chloride coefficient of NaOCl.

Fourth: Feeling that the hydrogen ion concentration of the diluted disinfectant, into which the 0.1 cc. of virile culture is measured, might exert an appreciable influence upon the phenol coefficient, we set out to discover its magnitude. Since the sodium hypochlorite we used furnished in the final dilutions a pH range of 9.72 to 9.83, a quantity of M/10 buffer was prepared which consisted of Clarke and Lub's borate-KCl mixture. When diluted to M/20 this mixture had a pH of 9.90. It served as a

buffer for the phenol, and held the pH of the latter in the dilutions used to pH 8.8 to 9.1. The influence of the buffer mixture itself upon the growth of B. coli (Bact. Neapolitanum) was predetermined under the conditions of the experimentations.

EXPERIMENTAL PROCEDURE

Reagents

The phenol used in this study was a quantity of Malinkrodt's c.p. which we redistilled from an all glass still. The first and last fractions were discarded. The standard 5 per cent solution was standardized against 0.1 N $Na_2S_2O_3$, following the bromate bromide procedure.

Mercuric chloride was obtained from Merck, "tested purity" and was recrystallized from conductivity water.

Sodium hypochlorite was prepared according to the method proposed by one of us (6). Pure chlorine from a drum was conducted into a solution consisting of 5 per cent of NaOH and 2.5 per cent of anhydrous Na_2CO_3 until the final concentration of "available chlorine" was 2.0 per cent. This solution was kept throughout the entire series of studies, which lasted during three months. It was not necessary to screen the hypochlorite solution from the light. A master dilution was made up from this 2.0 per cent solution which exacted 0.25 per cent of "available chlorine." The pH of the master dilution approached 10.1. This master dilution was made fresh preceding each disinfecting set.

The boric acid, NaOH and KCl were purified according to the well known procedure of Clark and Lubs.

Thermostats

The temperature for holding the disinfecting solutions at transfer was maintained at 20°C., ±0.1 in a circulating water bath electrically controlled.

A Freas electrically controlled incubator was used for incubating the inoculated broth tubes. A 37.5°C. temperature was maintained for all organisms whose optimum growth lay in this region.

Cultures of organisms

It was mentioned that pure cultures of these organisms had been obtained from reliable sources. Transfers of these organisms to sterile agar slopes were made every two weeks. A tube of the standard culture medium recommended by the Hygienic Laboratory (with our modification) was inoculated with the culture of the specific organism three days before the disinfection study, and another transfer was made twenty hours before using. It should be stated here that Bacto peptone (Difco) was substituted throughout for Armour's product.

The medium for the tubercle bacilli was composed of: 1 per cent Bacto peptone; 0.2 per cent NaCl; 0.3 per cent $Na_2HPO_4 \cdot 2H_2O$; 0.5 per cent of lipoid medium (supplied by Mr. Dunham of the Digestive Ferments Company, which courtesy is hereby acknowledged). Inasmuch as this organism is a surface grower, possessing a waxy coat, it is essential that all clumps be broken up and brought into a suspension. The cultures before transfer were therefore forty-eight hours old, and the tubes thoroughly shaken by tapping while being held between the thumb and forefinger to serve as a rocker. Two or three minutes' tapping brought the culture into the form of a homogenous suspension. Transfer from the disinfecting tubes showed that the 0.1 cc. of tuberculosis culture carried a goodly supply of the living cells into the disinfectant. It may be said in passing that the tuberculosis series was a very beautiful experiment in disinfection, both from the standpoint of sharp detail and esthetics.

The anthrax cultures were treated in the same manner as the tubercle bacilli, but were grown in the standard medium.

All other cultures, typhoid, coli, proteus, alkaligines and gonococcus, were treated in the regular manner. The gonococcus was grown in the same type of medium used for the tubercle organisms.

EXPERIMENTAL RESULTS

We have remarked that the limits of dilution chosen yielded in the majority of instances a phenol coefficient of large magnitude. In fact it is much greater in value than any previously

quoted. Repeated checks on the phenol coefficients of each organism agree in all detail so that the values expressed herewith are considered consistent with the dilutions and conditions used. By choosing intermediate dilutions and calculating interpolated phenol coefficients, it can be seen that the magnitude of the coefficient can be reduced about 20 per cent the order would not be changed.

TABLE 1

Phenol coefficient of NaOCl. Set No. 6 organism typhoid (Hopkins) pH 7.4 of culture

Date July 24, 1922. Checked July 27, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
			Phenol		
per cent					
2.5	−	−	−	−	−
2.0	−	−	−	−	−
1.5	+	+	−	−	−
1.0	+	+	+	+	+
0.5	+	+	+	+	+
			NaOCl		
0.010	−	−	−	−	−
0.0075	+	−	−	−	−
0.0050	+	+	+	+	−
0.00375	+	+	+	+	+

$$\text{Calculations for coefficient} = \frac{\frac{2.0}{0.01} + \frac{1.5}{0.0075} + \frac{1.5}{0.005}}{2} = 233.3$$

By way of illustration we are giving herewith the results of a single test to show how the dilutions and time of exposure to disinfectant relate.

It is apparent that if we had made dilutions of NaOCl intermediate between 0.0075 and 0.005 per cent we could have obtained a better slope of rate in our table. The same may be said of the phenol dilutions. It is doubtful if this is very important in fixing the relative power of disinfection.

TABLE 2

ORGANISM	PHENOL COEFFICIENT (APPROXIMATELY)
Tubercle bacilli	42.8
Alkaligines	100
Anthracis	160
Bact. Neapolitanum (coli)	200
Gonacoccus	280
Proteus	330

TABLE 3

Disinfecting power of M/20 borate potassium chloride buffer Organism—Bact. Neapolitanum (coli)

Temperature 20°C. pH, 9.91. July 31, 1922. August 1, 1922.

DILUTION BUFFER	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
M/20	–	–	–	–	–

TABLE 4

Influence of H ion concentration upon the phenol coefficient of NaOCl. Organism Bact. Neapolitanum

pH of culture, 7.4. Temperature 20°C. August 1, 1922. Concentration of buffer in final dilutions M/20 (borate—KCl).

DILUTION	TIME OF EXPOSURE					pH ELECTROMETRICALLY
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes	
Phenol in buffer						
2.0	–	–	–	–	–	8.80
1.5	–	–	–	–	–	8.98
1.0	+	+	+	+	+	9.08
0.5	+	+	+	+	+	9.12
NaOCl						
0.0075	–	–	–	–	–	9.83
0.005	+	+	+	+	–	9.76
0.004375	+	+	+	+	+	9.73
0.00375	+	+	+	+	+	9.72

Coefficient = 200 (approximately).

The phenol coefficients as determined for the other organisms are shown in table 2.

The pH of the diluted hypochlorite solutions used in the exposure tubes lay between 9.5 and 9.8. That of the phenol pH 7.0 to 7.2.

The points were determined colorimetrically after checking the buffer comparison mixtures electrometrically. Just how great an influence this great difference in hydrogen ion concentration exerts upon the disinfecting ratio of NaOCl and phenol

TABLE 5

Phenol coefficient of NaOCl without buffering the phenol. Organism Bact. Neapolitanum

pH culture, 7.4. Temperature 20°C. July 27, 1922.

DILUTION	TIME OF EXPOSURE					pH COLOR
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes	
			Phenol			
2.0	−	−	−	−	−	7.0
1.5	−	−	−	−	−	7.1
1.0	+	+	+	+	−	7.2
0.5	+	+	+	+	+	7.2
			NaOCl			
0.0075	−	−	−	−	−	9.8
0.005	+	+	+	+	−	9.75
0.004375	+	+	+	+	+	9.7
0.99375	+	+	+	+	+	9.7

Calculated coefficient (phenol) = 200 (approximately).

can be seen from the results given in table 3. We chose to work on Bact. Neapolitanum because of the large phenol coefficient observed and the ease of propagation of this organism. The buffer is not toxic to the above organism in the concentration employed.

It would seem that the pH of the disinfectant solution throughout the ranges employed has practically no effect upon the vitality of the living cell during this short exposure to its action.

TABLE 6

Relative disinfectant action of $HgCl_2$ and NaOCl Organism Bact. Neapolitanum

pH of culture, 7.5. Temperature 20°C. August 3, 1922. Checked August 4, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
$HgCl_2$					
0.025	−	−	−	−	−
0.01	+	−	−	−	−
0.0075	+	+	+	−	−
0.0050	+	+	+	+	+
NaOCl					
0.0075	−	−	−	−	−
0.0050	+	+	−	−	−
0.004375	+	+	+	+	−
0.00375	+	+	+	+	+

Calculated $HgCl_2$ coefficient = 2.3 (approximately).

TABLE 7

Phenol coefficient of NaOCl on tubercle bacilli. Organism tubercle

Age of culture, forty-eight hours. Temperature 20°C. Time of incubation at 37.5° of transfer, sixty hours. December 12, 1922. Checked December 8, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
Phenol					
2.0	−	−	−	−	−
1.5	+	−	−	−	−
1.3	+	+	+	+	−
1.15	+	+	+	+	+
NaOCl					
0.05	−	−	−	−	+
0.035	−	−	−	+	−
0.025	+	+	+	+	+
0.015	+	+	+	+	+

Calculated coefficient = 42.8 (approximately).

The mercuric chloride coefficient of NaOCl

When $HgCl_2$ is used in place of phenol on Bact. Neapolitanum we observe that sodium hypochlorite is slightly superior. The results of a preliminary series with these two substances gave the results in table 6.

Effect of a wax capsule on the disinfectant action of NaOCl

Virile forty-eight-hour cultures of tubercle bacilli were transferred to phenol and NaOCl exposure tubes at 20°C. The lipoid medium was used.

It is evident that there is a growth lag in case of the tuberculosis and sodium hypochlorite. It may be that the apparent growth lag is really a case of irregular quantities of the exposed material obtained by means of the platinum wire loop (spiral) at the time of transfer.

DISCUSSION OF RESULTS

While the coefficients obtained are large we feel that they are indicative of the phenol coefficient of sodium hypochlorite. We trust that some will be tempted to prepare sodium hypochlorite in accordance with our method, and repeat these experiments. The value of sodium hypochlorite has been both overestimated and underestimated. We actually need much scientific data on the subject. In the paper which follows we have shown that under certain conditions sodium hypochlorite is almost valueless as a bactericidal agent (i.e., in high protein solutions such as milk), yet even here it works with tremendous rapidity.

Our results seem to show that there may be some relationship between the reduction potential of bacterial cells and the toxic action (bactericidal) of sodium hypochlorite. But this needs further confirmation. One can expect that if there is a quantity of reducing material carried into the exposure tubes by the 0.1 cc. of virile culture, that such would require a comparatively large quantity of NaOCl to oppose it—to oxidize it. Likewise, if much protein material is carried over it would require much NaOCl for chlorination. However, the use of the same medium for each organism, and carefulness of transfer measurements,

should render this factor quite constant. Reduction potentials of the cultures used have been measured with a bare gold electrode, according to the method of Clark; likewise, reduction potentials of the exposure solutions have been measured, but these will not be discussed at this time. It is only in dilution experiments that any such exo-cellular or endo-cellular reduction effects can be determined.

The influence of small changes in pH on the phenol coefficient of sodium hypochlorite is not important in face of such widely different types of disinfectants.

From the standpoint of promiscuous use, sodium hypochlorite is a far better and safer disinfectant for the pathological laboratory, hospital and clinic than mercuric chloride.

We have become accustomed to the use of antiformin (equal parts of 15 per cent $NaOH$ and dilute $NaOCl$) for tuberculosis detection. The fact that $NaOCl$ has such a high phenol coefficient on tuberculosis organisms requires some comment. Doubtless the action of antiformin is largely dependent upon the strong alkalinity of the solution. The alkali serving to dissolve and hydrolize the protein matter in the sputum. The small quantity of $NaOCl$ merely acting as catalytic agent in the sense of hastening the action of the alkali on the bacterial cells. The tubercle cell, because of its thick covering of wax, resists the action of the alkali for a short period. Smears containing tuberculosis organisms and sputum were treated with 15 per cent $NaOH$ and then stained as in the antiformin method. Other smears were prepared containing the regular antiformin mixture and with $NaOCl$ in varying proportions. The results showed that the dissolving action of antiformin is primarily due to the free alkali. The presence of the $NaOCl$ actually shortened the time of solution. Likewise the hypochlorite must actually kill many of the tuberculosis organisms, although some may survive the treatment. It is not likely that any other organisms in the sputum could survive the treatment with antiformin.

SUMMARY

1. The phenol coefficient of stable sodium hypochlorite is very high, varying between 42 and 300 with the organisms employed.

2. The hydrogen ion concentration of the disinfectant in the exposure tube has little or no influence upon the phenol coefficient of NaOCl throughout the ranges employed.

3. Mercuric chloride is not as good a bactericidal agent as sodium hypochlorite.

4. NaOCl has a high phenol coefficient on the tubercle organism. The value of antiformin depends largely upon its content of alkalinity.

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THE BACTERIAL CONTENT OF ICE CREAM[1]

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INTRODUCTION

The phenomenal development of the ice cream industry is shown by statistics recently released by the Dairy Division of the United States Department of Agriculture. The total value of ice cream produced has increased 281 per cent in the seven-year period, 1914 to 1921. The ice cream made in 1914 was valued at $55,983,000 and in 1921 at $213,262,000. The consumption of ice cream in the United States in 1909 was 80,000,000 gallons, and in 1920 there were 260,000,000 gallons consumed, an increase of 225 per cent. This large increase is no doubt due to a better appreciation of its high nutritive value as well as its cooling effect.

With the increased demand for ice cream, there have come modern methods of manufacture and control. Laws have been passed in several states regulating the percentage composition and in some instances requiring pasteurization of the ice cream mix. A few attempts have been made to regulate the bacterial content of ice cream. This has not been entirely successful due to the limited knowledge of what a fair bacterial standard for ice cream ought to be. Unlike butter and cheese, ice cream is not so dependent upon bacteria for its flavor, and, except for the use of starters in recent years, microörganisms apparently have no function in the manufacturing process. This fact, together with the low temperature which practically inhibits bacterial growth, has led to the more or less prevalent but erroneous idea that bacteria are of no concern to the ice

[1] Contribution No. 61 from the Department of Bacteriology and No. 48 from the Department of Dairy Husbandry of the Kansas Agricultural Experiment Station.

cream maker. On the contrary the writers believe that the number of bacteria in ice cream is of vital concern both to the manufacturer and the consumer. As will be pointed out later, ice cream that has been made from clean raw products, and properly handled will contain relatively few bacteria. On the other hand ice cream that has been made from a poor grade of raw products, in unclean utensils, or has been improperly cared for during the manufacturing process will contain many bacteria and is undesirable from a sanitary point of view.

One of the most satisfactory methods of demonstrating the care with which ice cream has been produced is by determining the number of bacteria in it. When ice cream is found to contain 50,000,000 bacteria per gram, it may be safely assumed that it is not a desirable product to serve to sick people, to children, or even to healthy adults. An exception to this would be found in ice cream that has been ripened with a pure culture starter. On the other hand ice cream that contains 5000 bacteria per gram has more than likely been produced under carefully controlled conditions and from the best grade of raw products obtainable. The bacterial count, therefore, serves to differentiate between these two extremes. It is easy enough to say that ice cream containing 50,000,000 bacteria is undesirable, and one containing 5000 bacteria per gram is desirable from a sanitary standpoint, but it is not so easy to determine at what point between these two extremes a line of demarcation should be drawn. The present status of what is known about the bacterial content of ice cream does not justify the establishment of such a standard.

REVIEW OF LITERATURE

Although ice cream was probably first made about 1750 in Naples, it has not received attention by bacteriologists and public health officials until recent years.

In 1907, Pennington and Walter (18) reported a bacteriological study of commercial ice cream in Philadelphia. They classified 60 ice cream plants as follows: clean, 20; fair, 26; dirty, 6; and filthy, 8. They found a definite relation between the

cleanliness of the plant and the total number of bacteria in the finished product, but no relation between cleanliness and the number of streptococci. The maximum number of bacteria found in this investigation was 151,200,000 per cubic centimeter, and the minimum 50,000 per cubic centimeter. In one plant in which extraordinary sanitary precautions were taken, the bacterial counts of the finished ice cream in three successive trials were 6,535,000, 33,120,000 and 20,550,000 respectively.

In the same year Stiles and Pennington (23) examined 263 samples of ice cream in Washington, D. C., and found an average of 26,000,000, a maximum of 365,000,000 and a minimum of 137,500 bacteria per cubic centimeter. An investigation of 53 ice cream manufactories in Washington showed that 62.2 per cent were making ice cream in cellars and basements which were unfit for the production of such food. The 53 plants were classified as follows: clean 5.6 per cent; dirty 35.8 per cent; fair 30.1 per cent; and filthy 16.9 per cent. These authors believe that the cream and milk supply must be bettered before much improvement can be expected in the number of bacteria in ice cream.

Wiley (25) states that bacterial standards should be devised which would "exclude from sale ice cream containing a bacterial flora of the enormous proportions exhibited by some of the samples examined."

Buchan (5), in 1910, made a study of 50 ice cream venders (33 English and 17 Italian) and found "that most of the ice cream was not produced under sanitary conditions." Buchan's investigation involved an inquiry into the methods of preparation of the ice cream, a sanitary inspection of the premises, and a bacteriological study of the mix at various stages in the manufacturing process. He classed 10 per cent of the premises as clean, 36 per cent as fair, 46 per cent as dirty, and 8 per cent as filthy. The average bacterial count for 38 samples was 372,000,000 per cubic centimeter, the lowest count for 50,000 and the highest 3,800,000,000. Five of the samples, or 13 per cent contained over 1,000,000,000 organisms per cubic centimeter. Buchan concluded from his study that ice cream should

not be offered for sale if it contained more than 1,000,000 organisms capable of growing on nutrient agar (reaction) + 1 per cent at 35° to 37°C. in two days. This standard is quite lenient when compared to the requirements established by the Board of Public Health of the State of Victoria (22) in 1906, that ice cream shall contain not more than 50,000 organisms per cubic centimeter.

Newman (16) has pointed out that the frequent recurrence of epidemics due to ice cream in English cities led to the establishment of strict regulations by Liverpool in 1895, London in 1904 and by Glasgow in 1905. At the present time the city of Boston maintains a bacterial standard of 1,000,000 per cubic centimeter for ice cream.

In 1914, Bahlman (3) reported the results of investigations relating to ice cream in Cincinnati. An examination of 67 samples in 1911 showed a minimum of 200,000 and a maximum of 400,000,000 bacteria per cubic centimeter. In 1914, after the passage of an ordinance in Cincinnati requiring pasteurization of the cream, bacterial counts on 39 samples of ice cream showed a minimum of 60,000, and maximum of 35,000,000 per cubic centimeter. By pasteurizing the ice cream mix at 143° to 146°F. for thirty minutes, and carefully cleaning and steaming all utensils, Bahlman was able to produce ice cream containing no more than 12,000 bacteria per cubic centimeter. He concluded that if ice cream is properly pasteurized it would be very easy to comply with a legal standard limiting the bacterial content to 500,000 per cubic centimeter.

Ayres and Johnson (1) in 1915 collected 91 winter samples and 94 summer samples of ice cream from retail stores in Washington. None of these samples contained less than 100,000 and only 9.5 per cent contained less than 500,000 bacteria per cubic centimeter. The average for the 185 samples was 37,000,000; the maximum 510,000,000 and the minimum 120,000 per cubic centimeter.

In 1917 Hammer (10) made a comprehensive study of ice cream, including an investigation of the source of bacteria, the possibilities of securing a low bacterial count, and

the changes in the bacterial flora that take place during storage at low temperature. He concluded that unpasteurized cream is the greatest source of bacteria, that gelatin may or may not be responsible for large numbers, and that sugar, and vanilla extract are unimportant as a source of contamination. His investigations, also showed the importance of the utensils as a source of bacteria. By pasteurizing cream at 60°C. for twenty minutes, and sterilizing all utensils in an autoclave, he was able to produce ice cream in 15 and 20 gallon lots containing as few as 4200 bacteria per cubic centimeter.

Hammer and Goss (11) examined 13 samples of ice cream, other than vanilla, and found the bacterial counts to range between 130,000 and 40,000,000 per cubic centimeter. Water-ices and sherbets were found to contain from 6 to 6800 bacteria per cubic centimeter.

Ayers and Johnson (2) in 1917, made a study of the accuracy of bacterial counts on ice cream. By removing samples from nine places in various parts of a one-gallon container they were able to obtain results that checked very well. The bacterial counts on 198 samples ranged from 7150 to 225,000,000 per cubic centimeter. Fifteen samples contained more than 1,000,000, 5 more than 50,000,000, 9 more than 25,000,000 and 3 in excess of 100,000,000 bacteria per cubic centimeter.

In 1921 Hammer (13) emphasized the importance of the employees in an ice cream plant as a potential source of contamination with pathogenic organisms.

Jordon (14), in 1922 reported on the bacteriological examination of 1940 samples of ice cream in Boston, covering a period of ten years. Approximately 40 per cent of the samples contained more than 1,000,000 bacteria per cubic centimeter.

In 1923 one of the writers (9) reported on 115 samples of ice cream, all of which has been pasteurized. The maximum count was 47,000,000, the minimum 1500, and the average 1,895,000 per grams. Approximately 9 per cent of the samples contained fewer than 5000, 50 per cent less than 100,000, and 85 per cent less than 1,000,000 bacteria per gram.

Since the preparation of this manuscript Fabian and Cromley (26) have published the results of their study of the influence of manufacturing operations on the bacterial content of ice cream. Their method of attack and the results obtained are remarkably concurrent with the findings reported in this paper.

EXPERIMENTAL

The purpose of the experiment of which this paper is a partial report is to study the factors affecting the bacterial count of ice cream, the possibilities of producing ice cream having a low bacterial content under commercial conditions, and ultimately to establish a bacterial standard. The principal work so far has related to the possibilities of producing ice cream of a low bacterial count. The investigation was carried on in a representative commercial plant rather than in the college laboratories. About 200 gallons of ice cream were made per day during the summer, the amount varying between 100 and 400 gallons. The equipment, the surroundings, the methods employed, and the source of raw materials were such as will be found in many small ice cream plants throughout the country. For this reason the results should be applicable to every day conditions as they are found in the average commercial plant.

Plant methods. The mixing vat used was a plain cream ripener of 300 gallon capacity. The vat was washed first with cold water, then with hot water and Wyandotte washing powder, and finally rinsed with hot water. Following this it was steamed with covers closed until the temperature reached 200°F. or over. It was then allowed to stand with covers closed for ten minutes. The aging vat, also a 300-gallon cream ripener, was washed and sterilized in the same manner as the mixing vat.

The homogenizer was thoroughly washed and steamed before and after using in about the same manner as the mixing vat. The dissembled parts, after washing and steaming, were protected from dust, and steamed again for five minutes when the machine was reassembled.

The freezer was washed with cold water to rinse out the adhering ice cream and to partially warm the freezer. This was

followed by two washings of hot water, the last one containing Wyandotte washing powder. The machine was then rinsed with clear, hot water and sterilized by steaming for five minutes with the hose entering the outlet gate.

Preparation of the mix. In preparing the mix the liquid ingredients including milk, cream, and water were placed in the vat together. The gelatin, skim milk powder, and sugar, in the dry powder form were added separately by sprinkling over the surface of the cold liquid. Steam was then turned on and the contents of vat vigorously agitated until thoroughly mixed.

Since the unsalted butter could not be mixed properly with the other ingredients at ordinary temperatures it was necessary to sample the butter and the mix without the butter separately in order to determine the bacterial count of the mix before pasteurization. It was found that the skim milk powder, gelatin and sugar were practically all in solution or suspension by the time the mix reached 110°F., and a fair sample could be obtained. This temperature for the short time involved (about ten minutes) was not considered sufficient exposure to affect the bacterial content materially. The butter in pieces no larger than 1 pound was added immediately after this sample was taken from the vat. By the time the mix reached 140°F. all the butter was melted and mixed with the liquid constituents. The bacterial count before pasteurization, therefore, is a calculated count. It is calculated from the bacterial count of the butter, together with the number of pounds used, added to the bacterial count of the mix excluding the butter.

Pasteurization. The mix was pasteurized in most instances at 150°F. for thirty minutes. In some cases other combinations of time and temperature were used for comparison. The pasteurizing vat and also the aging vat were equipped with a Tycos bi-record recording thermometer.

Homogenization. After the mix left the pasteurizing vat, it was homogenized at pasteurizing temperature by means of a Viscolizer, at 1500 pounds pressure.

Aging. From the Viscolizer the mix was pumped into the aging vat to be cooled. When it had cooled to about 70°F. a

ripening powder was added. The temperature of the mix was then reduced to below 40°F. (usually about 36°F.) and allowed to stand over night. During the aging process the temperature remained between 36°F. and 45°F. for six to eight hours and then gradually increased to 50°F. after ten to fourteen hours. Rarely did the thermometer register above 50°F. the next morning. In a few instances the aging process was extended to thirty-six or forty-eight hours. In these cases it was kept below 50°F. throughout the period.

Freezing. The freezer in use was a 40-quart Fort Atkinson brine freezer. A sample was taken from the freezer and stored for two days in a hardening room, maintained at a temperature between 0 and 8°F. Another sample was taken simultaneously for immediate analysis.

Bacteriological methods. The bacteriological analyses were made on a gravimetric basis. The purpose of using a gravimetric sample was to overcome the variation in volumetric samples of melted ice cream resulting from incomplete exhaustion of air, and the adherence of melted ice cream to the pipettes. In order to make all the counts comparable a 10-gram sample was weighed under aseptic conditions for all of the samples as well as for the ice cream samples. The counts, therefore, represent the number of bacteria *per gram.* The determination of total numbers of bacteria was made according to the plate method recommended by the American Public Health Association (21), Liebig's beef extract, and Difco peptone were used in the preparation of the agar.

An effort was made to determine the different kinds of organisms present. Serial dilutions were made by inoculating a series of tubes with dilutions ranging from 1 in 10 to 1 in 10,000-000. One set of Durham fermentation tubes containing one per cent lactose broth and brom-thymol-blue as an indicator were inoculated. Another series of tubes containing nutrient gelatin was also inoculated. The highest dilution showing acid and no gas in lactose broth after forty-eight hours at 37°C. was recorded as the number of acid producing types. Similarly, the highest dilution showing gas was recorded as the number of acid

and gas producing types. The gelatin tubes were also incubated forty-hours at 37°C., and then removed and placed in iced water. The highest dilution which failed to solidify was recorded as the number of liquefying types present. Obviously there is a very high per cent of error in results obtained from serial dilutions. The conclusions which may be drawn from data obtained in this manner must necessarily be very general.

Samples were taken throughout the routine of manufacture as follows: (1) from the liquid constituents of the mix, including cream, whole milk, and the water (if added); (2) the mix before pasteurizing, not including the butter; (3) the butter; (4) the mix after pasteurization and before homogenizing; (5) after homogenizing and before aging; (6) after aging fifteen to eighteen hours and before freezing; (7) from the freezer; and, (8) after two days storage in the hardening room. Other samples were taken at intervals of the gelatin, sugar, skim milk powder, etc. All liquid samples were taken with a sterile milk thief and placed in a sterile glass stoppered bottle. From the vats, samples were taken at eight different places to insure as representative a sample as possible. The butter and frozen ice cream were sampled with a sterile butter trier by removing a core from four or five places in the container.

EXPERIMENTAL RESULTS

Table 1 shows the total counts obtained from various steps in the process of manufacture. During the first eight runs the customs and practices of the plant were not altered. From the ninth to the twenty-eighth run the entire manipulation of the mix was supervised by the writers.

It will be observed that the bacterial counts of the mix before pasteurization are in many instances exceedingly high, there being ten cases in which it exceeded 10,000,000 per gram. The lowest count before pasteurization was 2,059,000 and the highest 67,500,000 per gram. In general, it may be seen that the bacterial count after the mix is pasteurized governs to a large extent the bacterial count of the finished product. The effect of the various manipulations during the manufacturing process will be taken up separately.

Pasteurization. Table 2 shows the counts before and after pasteurization, the decrease in numbers of organisms, and the

TABLE 1

The total bacterial counts at various stages of the manufacture of ice cream

EXPERIMENT NUMBER	MIX BEFORE PASTEURIZING (CALCULATED)	MIX AFTER PASTEURIZING	AFTER HOMOGENIZING	BEFORE AGING	BEFORE FREEZING AND AFTER AGING	AFTER FREEZING	AFTER HARDENING FORTY-EIGHT HOURS
1	12,031,000	170,000	233,000	120,000	18,500		
2	3,398,000	500,000	160,000	60,500	44,000	56,000	67,500
3	6,028,000	285,000	128,000	250,000	262,000	188,000	107,500
4	6,306,000	58,000	52,500	39,500	35,500	52,500	119,000
5	2,477,000	75,500	120,000	79,000	79,500	97,000	102,500
6	26,147,000	2,210,000	2,830,000	3,700,000	3,835,000	2,880,000	1,530,000
7	31,100,000	35,500	111,000	95,000	244,000	1,165,000	1,080,000
8	9,328,000	124,000	132,000	178,500	165,500	900,000	1,315,000
9	38,985,000	27,000	270,000	11,000	10,000	24,000	30,000
10	22,053,000	70,000	109,000	160,000	41,000	97,000	66,000
11	67,500,000	2,070,000	153,000	111,000	115,500	160,000	21,000
12	29,360,000	44,000	2,980,000	23,000	27,000	64,000	22,000
13	2,059,000	6,500	11,000	9,000	8,000	20,000	13,000
14	44,774,000	20,000	20,000	14,000	12,000	11,000	7,000
15	6,197,000	52,000	19,500	73,000	108,000	172,000	140,000
16	46,103,000	27,000	63,000	152,000	70,000	150,000	115,000
17	6,898,000	32,000	36,000	16,000	63,000	67,000	35,000
18	9,685,000	42,000	44,000	43,500	10,500	26,000	19,000
19	2,290,000	13,000	16,000	14,000	28,000	22,000	23,000
20	5,634,000	51,000	47,000	54,000	48,000	42,000	34,500
21	4,236,000	70,000	53,000	73,000	33,000	31,000	30,000
22	2,551,000	5,700	7,000	5,200	9,800	10,000	7,400
23	6,803,000	19,300	27,600	11,000	14,000	29,500	11,800
24	53,386,000	6,400	9,500	8,200	6,300	7,300	30,500
25	5,219,000	9,850	12,200	11,400	8,400	11,650	9,650
26	12,630,000	26,000	84,000	25,400	16,300	18,650	24,150
27		60,000	12,000	7,700	8,350	12,000	8,150
28	2,685,000	49,000	29,000	25,000	65,000	77,000	62,000
Average.	17,261,926	219,953	277,475	191,782	192,362	236,688	186,320*

* The average bacterial count of the finished product in experiments 2 to 8 inclusive was 617,357; the average for experiments 9 to 28 inclusive when more care was taken in the manufacturing operation was 35,432 bacteria per gram.

per cent efficiency of pasteurization. The last two columns show the time and temperature used in this process for each of the runs.

The average of the counts before pasteurization in the 28 experiments was 17,261,926 per gram, and after pasteurization 219,953 per gram. This gives an average pasteurization ef-

TABLE 2

The effect of pasteurization on the total number of bacteria in ice cream

EXPERIMENT NUMBER	MIX		PER CENT EFFICIENCY	PASTEURIZATION	
	Before pasteurization	After pasteurization		Temperature	Time
					minutes
1	12,031,000	170,000	98.587	140	20
2	3,598,000	500,000	86.104	147	30
3	6,028,000	285,000	95.273	143	30
4	6,306,000	58,000	99.081	142	30
5	2,477,000	75,500	96.952	144	25
6	26,147,000	2,210,000	91.548	144	25
7	31,100,000	35,500	99.886	144	25
8	9,328,000	124,000	98 671	146	25
9	38,985,000	27,000	99.931	145	25
10	22,053,000	70,000	99.682	143	30
11	67,500,000	2,070,000	96.933	150	30
12	29,360,000	44,000	99.852	148	30
13	2,059,000	6,500	99.685	148	30
14	44,774,000	20,000	99.956	152	30
15	6,197,000	52,000	99.161	150	30
16	46,103,000	27,000	99.942	151	30
17	6,898,000	32,000	99.536	150	30
18	9,685,000	42,000	99.567	149	30
19	2,290,000	13,000	99.433	148	30
20	5,634,000	51,000	99.095	150	30
21	4,236,000	70,000	98.348	149	30
22	2,551,000	5,700	99.777	148	30
23	6,803,000	19,300	99.716	150	30
24	53,386,000	6,400	99.988	148	30
25	5,219,000	9,850	99 812	150	30
26	12,630,000	26,000	99.795	150	30
27		60,000		148	30
28	2,685,000	49,000	98.174	143	30
Average....	17,261,926	219,953	98.691		

ficiency of 98.69 per cent. In 17 cases the efficiency exceeded 99 per cent and in 4 cases more than 99.9 per cent. The fact that this work was done under practical conditions and with relatively

large volumes of mix may partly account for the comparatively lower pasteurization efficiency than has been obtained by Hammer and Sanders (12). By heating the mix to 145°F. and *not holding*, Hammer and Sanders were able to secure as high as 99.5 per cent reduction and an average destruction of 96.4 per cent of the organisms present. Tracy and Peterson (24) obtained better than 99.9 per cent reduction of bacteria by condensation of the mix. In the condensation process the mix is heated to 160° to 170°F. in the forewarmer, then condensed at 130°F. Bahlman (3) concluded from his work "that proper pasteurization and enforcement of regulations would reduce the bacterial content of ice cream, it would insure a safe product and give physicians some assurance of the safety of the product for invalids and children."

Examination of table 2 will show that there are 9 instances (experiments 1, 2, 3, 5, 6, 8, 11, 21 and 28), in which the efficiency of pasteurization is less than 99 per cent. In 6 of these cases (experiments 1, 3, 5, 6, 8 and 28) the temperature and time used for pasteurization did not comply with the laws of this state (Kansas) which require that all dairy products used in the manufacture of ice cream be pasteurized by heating to 145°F. for thirty minutes. In the other 3 of the 9 instances, (experiments 2, 11 and 21) the temperature and time used exceeded 145°F. for thirty minutes. However, if the data from experiments 2 and 11 be examined (table 1) it will be noted that the counts after pasteurization are apparently erroneous when compared to other counts obtained in the subsequent steps of the manufacturing process.

Further comparison of tables 1 and 2 will show that in six of the ten instances in which pasteurization did not meet the legal requirements, the bacterial content of the ice cream at the freezer was 100,000 per gram or greater. The data indicate very strikingly that the care of pasteurization is a primary factor in producing ice cream having a low bacterial count.

In all but two instances (experiments 15 and 16) in which 145°F. or a higher temperature for thirty minutes was used for pasteurization, the bacterial content of the finished product at

the freezer (column 5, table 1) was less then 100,000 per gram. Examination of table 1 will show that in these two exceptions the bacterial count of the mix after pasteurization was less than 100,000 per gram and that subsequent increase was apparently

TABLE 3

The total bacterial counts before and after homogenizing

EXPERIMENT NUMBER	MIX		INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
	Before homogenizing	After homogenizing				
1	170,000	233,000	63,000		37.0	
2	500,000	160,000		340,000		68.0
3	285,000	128,000		157,000		58.0
4	58,000	52,500		5,500		9.4
5	75,500	120,000	44,500		58.9	
6	2,210,000	2,830,000	620,000		28.0	
7	35,500	111,000	75,500		21.2	
8	124,000	132,000	8,000		6.4	
9	27,000	270,000	243,000		900.0	
10	70,000	109,000	39,000		55.7	
11	2,070,000	153,000		1,917,000		92.6
12	44,000	2,980,000	2,936,000		6672.7	
13	6,500	11,000	4,500		69.2	
14	20,000	20,000				
15	52,000	19,500		32,500		62.5
16	27,000	63,000	36,000		133.3	
17	32,000	36,000	4,000		12.5	
18	42,000	44,000	2,000		4.7	
19	13,000	16,000	3,000		23.0	
20	51,000	47,000		4,000		7.8
21	70,000	53,000		17,000		24.2
22	5,700	7,000	1,300		22.8	
23	19,300	27,600	8,300		43.0	
24	6,400	9,500	3,100		48.4	
25	9,850	12,200	2,350		23.8	
26	26,000	84,000	58,000		223.0	
27	60,000	12,000		48,000		80.0
28	49,000	29,000		20,000		40.8
Average.	219,953	277,475	57,520		26.1	

due to contamination. In experiment 15 the count is increased to above 100,000 per gram during the aging process, and in 16 the high count is apparently due to contamination from the

freezer. Therefore, these results, especially if considered in relation to the work of other investigators, indicate that if ice cream mix is pasteurized at 145° to 150°F. for thirty minutes, and the utensils do not offer a source of gross contamination, it is possible to manufacture ice cream under commercial conditions containing less than 100,000 bacteria per gram.

Homogenizing. Table 3 shows the effect of homogenization of the mix. There was an increase in the agar plate count 18 times and a decrease 10 times as a result of homogenizing. In general, however, the increases were larger than the decreases, and the averages of all the counts showed an increase of 26 per cent after homogenizing. Such an increase in bacterial count is no doubt more apparent than real, being due chiefly to the breaking up of clusters of organisms each individual of which may give rise to a separate colony on an agar plate. Hammer and Sanders (12) found that unless careful attention was paid to cleaning the homogenizer it would be a source of considerable contamination. It usually required from thirty to forty-five minutes to run the mix through the homogenizer, depending upon the quantity of mix that was made. Since homogenization was done at the temperature used for pasteurization, the last portion of the mix to go through the machine had been heated for an hour or more. This continued heating period made the efficiency of pasteurization even greater than is shown in table 2.

Aging. Table 4 shows the bacterial counts before and after the aging process. In 13 instances the number of organisms increased and in 15 cases there was a decrease. In half of the counts the increase or decrease is probably within the limits of experimental error.

The figures at the bottom of table 4 show that there was a difference of only 580 bacteria in the average counts before and after aging. This would indicate that in general the aging of the mix sixteen to twenty-four hours does not affect the bacterial count very markedly when the temperature is kept below 45°F. These results agree with those of Hammer (10) who found no increase in the count of the mix aged four days at

0°C. and also with those of Tracy and Peterson (24) who kept condensed mix for thirty-two days at 32° to 35°F. and found no marked increase in the number of bacteria until the fourteenth day.

TABLE 4

The total bacterial counts before and after aging

EXPERIMENT NUMBER	NUMBER OF HOURS AGED	BEFORE AGING	AFTER AGING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1	16	120,000	18,500		101,500		84.5
2	16	60,500	44,000		15,500		27.2
3	16	250,000	262,000	12,000		4.8	
4	16	29,500	35,500		4,000		10.1
5	16	79,000	79,500	500		0.6	
6	18	3,700,000	3,835,000	135,000		3.6	
7	16	95,000	244,000	149,000		156.8	
8	16	178,500	165,500		13,000		7.2
9	16	11,000	10,000		1,000		9.0
10	16	160,000	41,000		119,000		74.3
11	16	111,000	115,500	4,500		4.0	
12	16	23,000	27,000	4,000		17.3	
13	16	9,000	8,000		1,000		11.1
14	48	14,000	12,000		2,000		14.2
15	16	73,000	108,000	35,000		47.9	
16	15	152,000	70,000		82,000		53.9
17	24	16,000	63,000	47,000		293.7	
18	16	43,500	10,500		33,000		75.8
19	16	14,000	28,000	14,000		100.0	
20	16	54,000	48,000		6,000		11.1
21	24	73,000	33,000		40,000		54.7
22	16	5,200	9,800	4,600		88.4	
23	16	11,000	14,000	3,000		27.2	
24	16	8,200	6,800		1,900		23.1
25	16	11,400	8,400		3,000		26.3
26	16	25,400	16,300		9,100		35.8
27	16	7,700	8,350	6,500		8.4	
28	16	25,000	65,000	40,000		160.0	
Average..		191,782	192,362	580		0.3	

Freezing. The bacterial counts before and after freezing are shown in table 5. In order to eliminate the factor of contamination from the freezer, and to study the effect of the freezing operation on the bacterial count the samples were taken from the second and third freezing.

The data show an increase in the bacteria count as a result of freezing in 21 of the 27 samples with an average increase of 17 per cent. There were only six decreases, four of which (experiments 14, 19, 20 and 21) are probably within the limits of

TABLE 5

The bacterial counts before and after freezing

EXPERIMENT NUMBER	BEFORE FREEZING	AFTER FREEZING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1	18,500					
2	44,000	56,000	12,000		27.2	
3	262,000	188,000		74,000		28.2
4	35,500	52,500	17,000		47.8	
5	79,500	97,000	17,500		22.0	
6	3,835,000	2,880,000		820,000		22.1
7	244,000	1,165,000	921,000		377.4	
8	165,500	900,000	734,500		443.8	
9	10,000	24,000	14,000		140.0	
10	41,000	97,000	56,000		136.5	
11	115,500	160,000	44,500		38.5	
12	27,000	64,000	37,000		137.0	
13	8,000	20,000	12,000		150.0	
14	12,000	11,000		1,000		8.3
15	108,000	172,000	64,000		59.2	
16	70,000	150,000	80,000		114.2	
17	63,000	67,000	4,000		6.3	
18	10,500	26,000	15,500		147.6	
19	28,000	22,000		6,000		21.4
20	48,000	42,000		6,000		12.5
21	33,000	31,000		2,000		6.0
22	9,800	10,000	200		2.0	
23	14,000	29,500	15,500		110.7	
24	6,300	7,300	1,000		15.8	
25	8,400	11,650	3,250		38.6	
26	16,300	18,650	2,350		14.4	
27	8,350	12,000	3,650		43.7	
28	65,000	77,000	12,000		18.4	
Average.	192,362	236,688	44,326		23.0	

experimental error and are, therefore, insignificant. This concurs with the findings of Ellenberger (7) who states that, "aside from utensil contamination there is usually an increase in the number of bacteria resulting from the freezing process. This

is probably due to the breaking up of clumps of organisms." Hammer (13) agitated sterile water in a freezer and in five tests obtained counts as follows, 3700; 141,500; 8050; 1195; and 300 per cubic centimeter, thus illustrating the importance of the

TABLE 6

The influence of hardening on bacterial count of ice cream

EXPERIMENT NUMBER	BEFORE HARDENING	DAYS IN HARDENING ROOM	AFTER HARDENING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1							
2	56,000	2	67,500	11,500		20.5	
3	188,000	3	107,500		80,500		42.8
4	52,500	2	119,000	66,500		126.6	
5	97,000	2	102,500	5,500		5.6	
6	2,880,000	2	1,530,000		1,350,000		46.8
7	1,165,000	2	1,080,000		85,000		7.2
8	900,000	2	1,315,000	415,000		46.1	
9	24,000	2	30,000	6,000		25.0	
10	97,000	2	66,000		31,000		31.9
11	160,000	2	21,000		139,000		86.8
12	64,000	2	22,000		42,000		65.6
13	20,000	3	13,000		7,000		35.0
14	11,000	3	7,000		4,000		36.3
15	172,000	2	140,000		32,000		18.6
16	150,000	2	115,000		35,000		23.3
17	67,000	3	35,000		32,000		47.7
18	26,000	2	19,000		7,000		26.9
19	22,000	2	23,000	1,000		4.5	
20	42,000	2	34,500		7,500		17.8
21	31,000	2	30,000		1,000		3.2
22	10,000	2	7,400		2,600		26.0
23	29,500	3	11,800		17,700		60.0
24	7,300	2	30,500	23,200		317.8	
25	11,650	2	9,650		2,000		17.1
26	18,650	2	21,150	5,500		29.4	
27	12,000	2	8,150		3,850		32.0
28	77,000	2	62,000		15,000		19.4
Average..	236,688		186,320		50,368		21.2

freezer as a source of contamination. Hammer and Goss (11) found an increase in count after freezing in 84.3 per cent of their trials, the increase varying from 2 to 227 per cent.

Storage. Table 6 shows the difference in the total number of bacteria in the ice cream immediately after freezing and after forty-eight hours in the hardening room. There was an average decrease of 21 per cent.

The bacterial count decreased in 19 cases as a result of storage, and increased in 8 cases. It is improbable, however, that there was an actual increase in the number of organisms in any of these cases, due to the low temperature and short time of storage.

Stiles and Pennington (23) studied the changes in bacterial counts of four samples over a period of thirty-four days. In general the counts increased slightly between the first and third day, decreased gradually to the fourteenth day, then a marked increase occurred in all four samples on the twenty-seventh day followed by a sharp decline to below the original count on the thirtieth to thirty-fourth day. Esten and Mason (8) on the contrary were unable to find any such consistency in their study of 12 samples over a period of two months. Some of the samples increased and some decreased. They concluded, however, that there will be no marked increase or decrease in the bacterial content of ice cream stored for one month. Hammer (10) concluded that there was no tendency to increase the number of bacteria in ice cream on storage. Three of twelve samples which he examined showed a marked decrease in total numbers. Later Hammer and Goss (11) examined 39 samples of ice cream packed in salt and ice and 12 samples stored in a hardening room. Their findings indicate that during the proper storage of ice cream there is no increase in the number of organisms and there may be a decrease. The tendency to decrease apparently depends upon the bacterial flora of the ice cream. Ellenberger's (7) study of the changes in the bacterial content of ice cream in storage showed a slight decrease between the second and fourth day, after which there was a gradual decrease in viable bacteria. Ellenberger also showed that acid types (typically Bacterium lactis acidi) predominated throughout the period of storage.

This reduction in numbers may be due to the destruction of certain types of the less resistant varieties of bacteria present

in the ice cream. The low temperature of the hardening room would prevent any increase in such a short time as forty-eight hours. Therefore, the increases reported in table 6 are prob-

TABLE 7

Acid producers before and after pasteurization*

EXPERIMENT NUMBER	BEFORE PASTEURIZATION	AFTER PASTEURIZATION	DECREASE	PER CENT DECREASE
1	5,000,000	1,000,000	4,000,000	80.0
2	5,000,000	100,000	4,900,000	98.0
3	10,000,000	1,000,000	9,000,000	90.0
4	1,000,000	100,000	900,000	90.0
5	10,000,000	100,000	9,900,000	99.0
6	10,000,000	500,000	9,500,000	95.0
7	5,000,000	10,000	4,990,000	99.8
8	1,000,000	10,000	990,000	99.0
9	100,000,000	50,000	99,950,000	99.9
10	10,000,000	250,000	9,750,000	97.5
11	10,000,000	10,000	9,990,000	99.9
12	500,000	1,000	499,000	99.8
13	10,000,000	5,000	9,995,000	99.9
14	10,000,000	1,000	9,999,000	99.9
15	5,000,000	10,000	4,990,000	99.8
16	10,000,000	250,000	9,750,000	97.5
17	1,000,000	10,000	990,000	99.0
18	1,000,000	50,000	950,000	95.0
19	5,000,000	10,000	4,990,000	99.8
20	10,000,000	100,000	9,900,000	89.0
21	100,000,000	250,000	99,750,000	99.7
22	100,000,000	10,000	99,990,000	99.9
23	1,000,000	50,000	950,000	95.0
24	10,000,000	1,000	9,999,000	99.9
25	5,000,000	5,000	4,995,000	99.9
26	5,000,000	5,000	4,995,000	99.9
27	1,000,000	250,000	750,000	75.0
28	500,000	5,000	495,000	99.0
Average.......	15,785,714	147,964	15,637,750	99.0

* The term acid producer refers to the types that produce acid and no gas in lactose.

ably due in part at least to experimental error and not to an actual increase in the number present. The reduction in numbers varies with each different mix and is shown in table 6 to

range from 7 to 86 per cent. Such variation may be due to a difference in the number of the types present possessing low resistance to freezing temperatures.

TABLE 8

Acid and gas producers before and after pasteurization*

EXPERIMENT NUMBER	BEFORE PASTEURIZATION	AFTER PASTEURIZATION	DECREASE	PER CENT DECREASE
1	1,000,000	0	1,000,000	100.000
2	250,000	0	250,000	100.000
3	100,000	0	100,000	100 000
4	50,000	0	50,000	100.000
5	5,000,000	0	5,000,000	100.000
6	50,000	0	50,000	100.000
7	1,000,000	0	1,000,000	100.000
8	100,000	0	100,000	100.000
9	5,000,000	0	5,000,000	100.000
10	1,000,000	0	1,000,000	100.000
11	10,000,000	100	9,999,900	99.999
12	500,000	0	500,000	100.000
13	500,000	500	499,500	99.900
14	1,000,000	10	999,990	99.999
15	500,000	10	499,990	99.998
16	10,000,000	10	9,999,990	99.999
17	500,000	0	500,000	100.000
18	500,000	10	499,990	99.998
19	100,000	0	100,000	100.000
20	10,000,000	0	10,000,000	100.000
21	500,000	0	500,000	100.000
22	5,000,000	10	4,999,990	99.999
23	1,000,000	10	999,990	99.999
24	10,000,000	10	9,999,990	99.999
25	500,000	100	499,900	99.998
26	1,000,000	500	999,500	99.950
27	1,000,000	0	1,000,000	100.000
28	5,000	1,000	4,000	80.000
Average.......	2,362,678	81	2,362,597	99.996

* The term acid and gas producer refers to the types that produce acid and gas in lactose.

The effect of low temperatures on bacterial cells has been studied by MacFadyean and Rowlands (15), Park (17), Belli (4), Conn and Esten (6), Pennington (19), Ravenel, Hastings and

Hammer (20), and many others. Hammer (13) demonstrated that the low temperature at which ice cream is held can not be relied upon to destroy pathogenic organisms. Ice cream

TABLE 9

Gelatine liquefiers before and after pasteurizing

EXPERIMENT NUMBER	BEFORE PASTEURIZING	AFTER PASTEURIZING	INCREASE	DECREASE
1				
2				
3	500	500		
4	0	500	500	
5	0	1,000	1,000	
6	10	1,000	990	
7	100	500	400	
8	5,000	1,000		4,000
9	5,000	1,000		4,000
10	1,000	1,000		
11	0	100	100	
12	0	0		
13	0	500	500	
14	100	500	400	
15	0	100	100	
16	500	1,000	500	
17	100	100		
18	10,000	500		9,500
19	0	10	10	
20	100	1,000	990	
21	10,000	5,000		5,000
22	0	5,000	5,000	
23	0	100	100	
24	100,000	1,000		99,000
25	0	500	500	
26	100,000	5,000		95,000
27	0	5,000	5,000	
28	10	10		
Average.......	8,939	1,227		7,712*

* Average per cent decrease = 86.

that had been artificially inoculated with tubercular organisms was still infective for guinea pigs after one month of storage.

Types of bacteria in ice cream. The differentiation of the types of bacteria in ice cream was made on a basis of the action of the

organisms on lactose and on gelatin. Tables 7, 8 and 9 give the results of these differential counts on the samples before and after pasteurization. As previously described, the number of acid formers and the number of gas formers were determined by serial dilution of the sample in Durham fermentation tubes, containing lactose broth and brom-thymol-blue. The terms acid formers, and acid and gas formers, therefore, refer to the action of the organisms on lactose. The gelatin liquefiers were determined by serial dilution of the mix in nutrient gelatin. The highest dilution which failed to solidify in ice water after forty-eight hours incubation at 37°C. was taken as the number of liquefying types.

The number of acid forming types before and after pasteurization is shown in table 7. The average number of acid forming types before pasteurization was nearly 16,000,000 and only about 150,000 after pasteurization. There was no instance in which there were less than 1000 acid forming types per gram surviving pasteurization, thus illustrating the fact that pasteurization does not kill all of the acid producing types.

Pasteurization is very efficient, however, in destroying the gas producing types, as shown in table 8. In 16 of the experiments all of the gas producing types were killed and in seven instances there were only 10 gas producers per gram after pasteurization. The average number surviving was only 81 per gram. The number of these types in the raw mix varied from 5000 to 10,000,000 and averaged 2,362,000 per gram.

The gelatin liquefying types were not so consistently reduced by pasteurization. In 15 of the 26 experiments an increase was noted in the number of liquefying types after pasteurization. It will be observed in table 9, however, that the increases are relatively small and probably within the limit of the error of the method. Even though there may not have been an actual increase in the number of liquefying types, the data would indicate that in these 15 experiments at least, the number of gelatin liquefiers was not materially reduced. In 5 of the experiments there was a quite marked reduction in the gelatin liquefying types. These reductions were of sufficient magnitude

to make the averages of the 26 experiments show an 86 per cent decrease in the gelatin liquefying types after pasteurization. The average number of liquefiers was about 9000 per gram before, and 1200 after pasteurization.

Ayres and Johnson (1) made a qualitative examination of 71 samples of ice cream by the "milk tube" method. They found 30.84 per cent acid coagulating types, 38.03 per cent acid forming types, 5.42 per cent alkali forming types, 20.9 per cent peptonizing types, and 4.81 per cent of inert forms. Gas forming types were found in 0.1 cc. of 106 of the 120 samples examined.

SUMMARY

In this work an effort has been made to determine the possibilities of producing ice cream with a low bacterial content under commercial plant conditions. The conditions under which the work was done and the products used in some of the mixes were far from ideal. Care was taken not to employ methods to lower the bacterial count that were impractical or that could not be followed by any manufacturer.

Since the purpose of the work was to determine the possibilities of producing ice cream with a low bacterial count, a slightly higher temperature of pasteurization was employed in most of the experiments than is required by the state law. The data presented show that it is possible to produce ice cream consistently having less than 100,000 bacteria per gram, even though in many instances the raw mix contained several million per gram. It is also quite evident from the data that the bacterial count of the finished product is more dependent upon the temperature and time used in pasteurization than upon the original number of bacteria in the raw mix. In this respect the bacterial count of ice cream may not reveal the use of raw products of poor quality. It is possible for a manufacturer to utilize a high temperature of pasteurization to conceal the low grade of ingredients used in the mix. It is quite probable that some manufacturers would resort to such practices if bacterial counts were being determined on the ice cream. It would at least have the desirable effect of stimulating careful pasteurization.

One point repeatedly observed throughout the progress of the experiments was that any carelessness on the part of the ice cream maker invariably resulted in a high bacterial count of the finished product. If the pasteurization time or temperature were reduced, utensils not thoroughly cleaned, the temperature during aging was not properly controlled, a high count in the finished ice cream was almost certain to result. This observation was so consistent that it was even possible to predict a high or low bacterial count of the finished product from the time and care used in its manufacture.

The results in this paper on aging of the mix may not be applicable to those plants using a longer period of aging than was in use with this work. These results show practically no change in the bacterial count before and after aging except in a few instances. If these mixes had been aged three to six days, as is occasionally practiced, there would probably have been a slight increase in the number of organisms after aging. The temperature used for aging is such that bacterial growth is very slow and is negligible in fifteen to eighteen hours. When long aging periods are used, a slight increase may be expected, but if the temperature is properly controlled, this increase should not be sufficient to cause an excessively high count in the finished product. However, if the temperature is not well controlled and is allowed to raise only a few degrees the bacterial content will be greatly increased. Increasing the temperature ten degrees may mean an increase of several hundred per cent in the bacterial count after twenty-four hours. This increase in the bacterial content may be constituted of perfectly harmless types of bacteria, and the ice cream made from the mix may taste just as good as though the bacterial growth had been checked. It is the contention of the writers, however, that the failure to control the temperature during the aging process is indicative of careless methods and careless methods in turn indicate an undesirable if not an unsafe product. Hammer and Sanders (12) state that, "while in most ice cream of high bacterial content Bacterium lactis acidi, a harmless type, predominates, the entrance and rapid multiplication of this organism occurs under

conditions that make possible the entrance and multiplication of undesirable and possibly harmful types." Although it is true that ice cream having a high bacterial count due to a dirty plant, filthy utensils and poor raw products is much less desirable than a product having a high count due to growth of bacteria during the aging process, nevertheless it is difficult to determine to which factor the large number of bacteria is due.

This direct relation between the care of manufacture and the bacterial count indicates that a bacterial standard for ice cream would be particularly valuable. The increased use of ice cream emphasizes the necessity of controlling its manufacture. The consumer should be protected against the use of a product made under undesirable conditions. Since the bacterial count affords a fairly good check on the conditions surrounding the manufacture of ice cream, its use is recommended for such an index.

The problem of establishing standards is one that requires more data than is available at present. In this work ice cream has been consistently produced with less than 100,000 bacteria per gram by pasteurizing at 150°F. for thirty minutes. It remains to be shown what the bacterial count of ice cream should be when it is pasteurized at the minimum requirements of the present law, 145°F. for thirty minutes.

CONCLUSIONS

1. The temperature and time of pasteurization are the most important factors governing the bacterial count of ice cream.
2. A high bacterial content of the raw products used may be concealed by the use of high temperature of pasteurization.
3. The utensils need not be a source of gross contamination if they are properly washed and steamed.
4. Pasteurization at 150°F. for thirty minutes destroys practically all of the types of bacteria that produce acid and gas on lactose, but fails to destroy all of the types that produce only acid on lactose, or the types that liquefy gelatin.
5. Homogenization of the mix usually causes an increase in the bacterial count as determined by the agar plate method.

Such an increase is probably due to the breaking up of clumps of bacteria.

6. The aging of the mix for fifteen to eighteen hours at low temperature does not result in a material increase in the number of bacteria present.

7. As a result of the freezing process there is a slight increase in the agar plate count which is probably due to the breaking up of clumps of bacteria by the whipping action of the freezer.

8. There is a decrease in the number of bacteria during the first forty-eight hours of storage.

9. It is possible and practical to consistently produce ice cream containing less than 100,000 bacteria per gram by pasteurizing at 150°F. for thirty minutes and by using utensils only that have been thoroughly cleaned and steamed.

10. The bacterial content of ice cream serves as a good index to the conditions surrounding its production. Carelessness in handling the mix or cleaning the utensils will be revealed by a high bacterial count. Except in case of the use of a starter, ice cream containing large numbers of bacteria has been neglected at one or more steps in its manufacture, or it has been made from undesirable products.

11. The direct relation between the bacterial content of ice cream and the conditions under which it has been produced, suggests the value of establishing bacterial standards for this important food.

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THE RELATION BETWEEN YEASTS AND MOULDS AND THE KEEPING QUALITY OF BUTTER

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Much work has been done in the past few years to demonstrate that pasteurized creamery butter is often highly contaminated with microörganisms during the making. Lund (1) and others have ably discussed this subject. Little has been accomplished, however, in relating the actual keeping quality of the butter, so contaminated, over a period of six months in cold storage. That there is a definite relation existing between this contamination and the keeping quality, there is no doubt, but published data is scarce.

During the season of 1921, it was my privilege to be employed in the Bacteriology Department of the Ontario Agricultural College, on the yeast and mould analysis of butter for the Ontario Butter Grading Station. The following are extracts from a thesis prepared at that time by the writer, and may prove of interest.

In all 573 samples of butter were examined during the season. They consisted of a plug, taken with a sterile tryer from a 14-pound box and placed in a sterile jar for shipment to the laboratory. The samples were sent a distance of fifty miles over night and were plated the following morning. The usual plating methods were followed with the exception that wort agar (acidified) was used. Twenty-one of these samples were selected during the early part of the season and held in cold storage at 10°F. They were scored from time to time as directed, by the Official Grader. More boxes would have been set aside, but unfortunately good samples were scarce.

Preparation of wort agar

	per cent
Wort, cleared and filtered	40
Water, distilled	60
Agar, market	1.5

The wort was ordinary wort used in the manufacture of beer and was obtained from the Springbank Brewery, Guelph. It is known as unhopped wort. It was cleared, filtered and sterilized in the laboratory. Before pouring the plates, the wort agar was acidified with 5 per cent sterile lactic acid solution: 10 cc. of the solution to 250 cc. of melted wort agar.

TABLE 1

The effect of contamination by yeasts and moulds on the original scoring of pasteurized creamery butter, and on the scoring after long storage at 10°F.

SAMPLE NUMBER	MONTH	DAY	YEAST COLONIES	OIDIUM COLONIES	PENICILLIUM COLONIES	SCORE				
						Original	August 18	October 10	November 29	December 20
1	6	18	*	0	0	38	38	37.5	36	36
2	6	11	*	0	0	40	39	39	38	38
3	6	20	*	1	0	41	39	39.5	39	39
4	6	17	*	0	0	40.5	40	40	39.5	39
5	6	13	*	0	0	40	38.5	39	39	39
6	6	11	*	0	0	39	39	38	36.5	36
7	6	24	*	0	0	38		38	38	37.5
8	6	25	*	0	0	38		38.5	37.5	36
9	5	28	*	1	0	38.5		38	37	36.5
10	5	27	70	0	1	39	39.5	39.5	39	39
11	6	2	20	0	0	39.5	39.5	39	39	39
12	6	6	80	0	0	39.5		39.5	39	39
13	5	16	130	0	0	39		38.5	38	38
14	5	14	20	0	0	39		39	39	38.5
15	6	1	*	1,000	0	39	39.5	39	37.5	37.5
16	6	28	*	32,000	0	37.5	38.5	38	37	36
17	5	25	2,300	1,200	0	38	38	38	36	37
18	6	20	*	150	0	40		38.5	38	38
19	6	27	120	180	0	39.5		39	38.5	38
20	6	20	*	600	0	41		39.5	37	37
21	5	6	35,000	0	350	38	38	37.5	37	37

* Over 100,000 colonies per cubic centimeter.

Flavor standards at the Ontario Butter Grading Station, 1921

Minimum for special grade	Flavor 41
Minimum for grade 1	Flavor 39
Minimum for grade 2	Flavor 37
Off grade, below	Flavor 37

The effect of contamination by yeasts and moulds on the original scoring and on the later scorings will be found in table 1.

DISCUSSION OF TABLE I

The effect of high yeast count

It will be noted that there are 9 samples containing over 100,000 yeast colonies per cubic centimeter, accompanied with fewer than two moulds. One sample is a special, 4 are in grade 1, and 4 are in grade 2 when first scored. There is quite a difference between these samples in the scoring. The creamery with the special, turned out that class of butter most of the season, as did some of the other four in grade 1. In accounting for this, it would appear that these creameries possessed a desirable type of yeast that gave the butter at scoring a delicate aroma. Such yeasts are known. Unfortunately, however, the scoring of December 20 shows a drop in all 9 samples; 4 to off-grade.

The effect of low yeast count

There are 5 samples having less than 200 yeast colonies per cubic centimeter, coupled with fewer than two moulds. They are all in grade 1 when first scored. After approximately six months in storage, 3 remain in grade 1, 1 has dropped half a point and the other has dropped 1 point. They have all held their flavor to a greater degree than the high yeast samples. The high yeast samples ranging from half a point to three points loss in flavor. The half point mentioned in the high yeast samples being in grade 2 when first scored. The manager of one of these creameries reported that he was able to place his June butter on the market in February, in grade 1, without any loss.

The effect of mould

There are 7 samples containing over 100 mould colonies per cubic centimeter. Four are associated with over 100,000 yeast colonies. One with 2300, one with 120 and the other with 35,000, nos. 17 and 21 are in grade 2 when first scored. No. 19 is in grade 1, but drops to grade 2. Of the 4 accompanied with over 100,000 yeasts, 3 are in grade 1 when first scored. This is probably due to a favorable yeast. The moulds would most probably be in the spore stage and would not have had time to

germinate and form mycelium. It will be observed, however, on glancing at the final scoring that all three have deteriorated, the greatest occurring with no. 20 which has dropped from special grade to grade 2.

SUMMARY

Twenty-one samples of pasteurized creamery butter were selected and held in cold storage at 10°F. for a period of approximately six months. It would appear that butter made from pasteurized cream and churned with the least possible contamination will hold its grade to better advantage, than butter made from pasteurized cream and later contaminated during the churning process.

ACKNOWLEDGMENT

Thanks are due to the Department of Bacteriology, of the Ontario Agricultural College, Guelph, for the full use of the laboratory, and to the Ontario Butter Grading Station, Toronto for their coöperation in regrading the above samples.

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ADULTERATED ACID AS A POSSIBLE SOURCE OF ERROR IN TESTING MILK BY THE BABCOCK METHOD

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I. INTRODUCTION

Commercial sulfuric acid as used in making the Babcock test varies greatly in appearance and strength depending upon age, impurities contained and other substances with which it may have come in contact. Acid takes on a decidedly dark color with age and usually weakens in its activity. The same is true with acid with which bits of cork or other organic substances has come in contact. It is therefore customary to consider acid, dark in color, to be weak and the tester uses a larger quantity than is prescribed in order to get satisfactory results.

Due to the impurities that it may contain, fresh sulfuric acid may have a pink or yellow tint. Sulfuric acid is also usually kept in earthenware jugs from which small quantities are poured as it is needed. Therefore an abnormal color does not attract as much attention as would be the case if the acid would be seen in larger quantities.

These facts increase the possibility of the user overlooking adulterants which may have been added to the sulfuric acid for the purpose of increasing the fat reading if such adulteration only slightly affect the color of the acid.

II. OBJECT OF THE INVESTIGATION

The author's attention was called to the possibility of adulterating sulfuric acid when a milk tester encountered acid to which oil of apparently a high grade had been added. Use of this acid resulted in tests so high that they could not be read in ordinary 8 per cent test bottles.

The object of this investigation was twofold: first, to determine if it is possible, by adding an adulterant to the acid used, to increase the fat percentage as shown by the Babcock test; and second, if it is found possible to influence the reading in this manner how may the adulteration of the acid be detected?

III. METHOD OF PROCEDURE

Sulfuric acid is used in testing milk for butterfat because it reacts rapidly with all the solids of the milk with the exception of the fat upon which it has no effect except when too concentrated. This, together with the fact that the substance added to the acid would have to be recovered, in part or in whole, in the fat column of the completed test in order to effect the reading, would make it seem reasonable that the substances suitable for this experiment would be limited to: (1) fats or oil substances, (2) fat solvents and (3) a combination of these two. In this investigation the study of substances added to sulfuric acid were divided into these three groups, hereafter referred to as group I, group II, and group III.

Under group I, fats or oil substances, the following were studied: butterfat, lard, cottonseed oil, linseed oil, olive oil, corn oil, cod liver oil, sperm oil and several of the mineral oils. Under group II, fat solvents, the following were studied: gasoline, benzine, ligroin, xylol, kerosene and ether. Under group III, combinations of the substances listed in groups I and II, each of the solvents in group II were studied when saturated with the fats of group I.

The first object was to determine whether or not the substances as listed in the three groups would increase the reading of the milk if added to the sulfuric acid used. In case the result was positive it was planned to study the effect upon the acid as to color, consistency and activity, and the effect upon the fat column of the completed test.

The next step was to determine, for those substances found to increase the fat reading, the relation between the amount used as an adulterant in the acid and the fat reading.

For each substance tested as a possible adulterant for acid, a series of six samples were prepared. Each consisted of 200 cc. of commercial sulfuric acid and the amount of the adulterant added was: 0.5, 1, 1.5, 2, 2.5 and 3 per cent. From each of these adulterated lots of acid, ten successive portions of 17.5 cc. each were added to samples from the same lot of milk measured into Babcock test bottles in the usual manner. The tests were completed according to the usual Babcock method including duplicates in which normal acid was used.

The third point of the investigation was the possibility of detecting such adulteration of the acid by observing the appearances of the acid when adulterated, the looks of the fat column of the completed test and by running blanks made by adding the amount of acid regularly used in the test to water instead of milk.

IV. DISCUSSION OF RESULTS

Group I

All the substances in group I, i.e., butterfat, lard, cottonseed oil, linseed oil, olive oil, corn oil, cod liver oil, sperm oil, and the mineral oils gave similar results. A 2 per cent addition of each of these substances in sulfuric acid gave a marked increase in the fat reading, varying from an increase of 0.65 per cent acid-lard mixture to 1.25 per cent acid-sperm oil mixture. Adulteration of the acid by any of this group can readily be detected from the appearance of the acid. The color of the acid changes quickly to a dark wine color or becomes black and of a sirupy consistency when any of these substances are added, even in very small amounts. All of these substances rise to the surface of the acid within a few minutes after mixing, floating about in black masses. Because of this fact, duplicate samples seldom check in testing. The fat column is usually dark but in some cases of good color.

Group II

All substances of this group, i.e., gasoline, benzine, ligroin, xylol, kerosene and ether behaved alike in that they all floated

on top of the acid, and with the exception of kerosene and ether, they behaved alike in all other respects.

Table 1 shows that a 2 per cent addition of each substance of group II to the sulfuric acid increased the fat reading of the milk where such adulterated acid was used, in some cases more than 2 per cent, except in the case of ether where the effect was negative. It will be noted that in no case did the first and the second test of the milk check where the adulterated acid was used. It will also be noted that in some cases the increase in the reading was greater than the per cent of substance added to the acid. This is accounted for by the fact that all of these substances floated on the top of the acid and that in pouring off the acid into the acid measure more of the adulterant would be poured off in the first portion than in any of the subsequent ones.

TABLE 1

The effect of fat solvents as adulterants with sulfuric acid upon fat reading of milk where such acid is used in conduct of the Babcock test

ADULTERANT AND AMOUNT	FAT READING WITH ADULTERATED ACID		FAT READING WITH NORMAL ACID	
	First test	Second test	First test	Second test
	per cent	*per cent*	*per cent*	*per cent*
2 per cent gasoline	6.9	6.4	4.2	4.2
2 per cent benzine	8.0	7.9	6.4	6.4
2 per cent ligroin	5.0	4.7	3.2	3.2
2 per cent xylol	6.5	6.2	4.2	4.2
2 per cent kerosene	4.3	4.1	3.4	3.4
2 per cent ether	3.4	3.4	3.4	3.4

This table is typical of results secured in numerous trials. The size of the vessel in which the adulterated acid is kept and the rapidity with which the acid sample is poured are factors which make for a variation from the above.

Effect upon the acid. With the exception of kerosene, none of the substances of group II had any apparent effect upon the color or the consistency of the acid. Kerosene caused the acid to become of a greenish color that would arouse suspicion. The addition of any of these substances had no apparent effect upon the activity of the acid.

Effect upon the fat column. In addition to increasing the reading of the fat column, all of the substances of this group produced unusually clear fat columns. The fat column was of clear amber color, and in no case was a "burnt" fat column noticed.

Relation of the quantity of adulterant used to the increase in the fat reading. Of a large number of trials table 2 was selected as being typical of the results that were secured with the various

TABLE 2

Effect of gasoline as an adulterant of sulfuric acid

AMOUNT OF GASOLINE ADDED TO THE ACID	FAT READING OF SUCCESSIVE PORTIONS OF ADULTERATED ACID									
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.5	4.0	4.0	3.95	3.9	3.9	3.9	3.9	3.9	3.9	3.9
1.0	4.5	4.5	4.2	4.4	3.9	3.9	3.9	3.9	3.9	3.9
1.5	4.9	4.7	4.6	4.4	4.3	4.1	3.9	4.0	3.9	3.9
2.0	5.8	5.4	5.1	4.8	4.4	4.2	4.0	3.9	3.9	3.9
2.5	7.0	5.9	5.4	5.1	4.8	4.6	4.1	4.1	3.9	3.9
3.0	7.9	6.2	5.5	5.3	5.0	4.7	4.4	4.4	4.2	4.1
Normal acid	3.9	3.9								

Although duplicate trials did not check exactly, this table may be taken as typical results to be expected from additions of gasoline in varying amounts to sulfuric acid.

substances of this group. This table gives the fat reading secured by each of ten successive 17.5 cc. measures of gasoline-acid mixture. It will be seen that when the amount of gasoline added to the acid exceeds 2 per cent the first and second samples fail to check within the customary limit of 0.2 per cent. It will also be seen that with a 2 per cent addition of gasoline to the acid, each successive sample of adulterated acid poured off caused a decrease in the fat reading until the fifth or sixth portion when practically all of the gasoline had been poured off. In the case where the adulteration is 3 per cent of gasoline the fat reading was influenced up the tenth sample.

The apparent irregularity of the decrease in the fat reading with the different percentages of adulteration may be accounted

for by the difference in rapidity with which the acid was poured off into the acid measure. This would also account for the fact that in duplicate trials identical results were not secured. Results expressed in table 2, however, are fairly representative of results that may be expected.

Detecting the presence of group II. With the exception of kerosene none of the substances of this group can be detected readily by appearance. As they all float on top of the acid and are transparent, they are not noticed without careful examination unless present in relatively large quantities. The fact that if any of these substances are present in sufficient

TABLE 3

The effect of butterfat saturated solvents as adulterants

ADULTERANT	FAT READING OF SUCCESSIVE PORTIONS											
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Normal	Increase
2 per cent addition of saturated solution of butterfat in:												
Gasoline	6.4	6.4	6.4	6.3	6.4	6.4	6.4	6.4	6.4	6.4	5.2	1.2
Benzine	6.4	6.5	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	4.9	1.4
Xylol	4.4	4.4	4.4	4.5	4.4	4.4	4.4	4.4	4.5	4.4	3.7	0.7
Ligroin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	3.7	1.3
Ether	4.3	4.3	4.1	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	

Results are here shown of tests with ten successive portions of acid containing 2 per cent of a saturated solution of butterfat in each of the five solvents, gasoline, benzine, xylol, ligroin and ether. None of these additions effected the color of the acid, and with the exception of ether saturated with butterfat, all remained in a mixture with the acid for more than an hour after being thoroughly mixed.

quantities to influence any number of successive tests the first samples will not check within the permitted limits and will therefore arouse suspicion. Gasoline and benzine can be detected by odor and all of this group can be detected by running a blank test by the Babcock method where water is used in place of milk. These substances will then appear in the neck of the test bottle and the amount of addition can be determined.

Group III. Saturated solutions of fats in fat solvents

Saturated solutions of butterfat in either gasoline, benzine, ligroin, xylol or kerosene gave marked increases in the fat reading of milk when such saturated solutions were added to the acid used. A saturated solution of butterfat in ether increased the fat reading but slightly.

Effect upon the acid. Saturated solutions of these butterfat solvents effected the color of the acid very little when the acid was cool producing a very pale yellow. The coloration, however, was very slight. In a practical test an experienced milk tester preferred such adulterated acid to that of six months old acid which had turned dark in color. When these saturated solutions were added to warm acid the coloration was more marked. It was also noted that these saturated solutions remained in mixture with the acid for some time after being mixed. After standing for several hours they would rise to the top of the acid, but when agitated, would again go into a mixture with the acid. A saturated solution of butterfat in kerosene caused the acid to turn green. When ether and butterfat solution was added to the acid there seemed to be a reaction between the ether and the acid that liberated the butterfat which then floated on top of the acid in charred masses Saturated solutions of the other fats and oils in these solvents caused a much more marked coloration of the acid.

The activity of the acid seemed not to be affected by the adulteration of any of these butterfat saturated solvents except that upon mixing milk and such adulterated acid, bubbles seemed to be formed immediately after the mixing was completed. This however, would not be noticed unless especially looked for.

Effect upon the fat column. Acid adulterated with saturated solutions of butterfat in gasoline, benzine, ligroin, kerosene or xylol produced unusually clear fat columns of amber color. The higher the percentage of adulteration the lighter in color was the fat column.

Uniformity of results. Table 3 was selected as expressing typical results found in numerous trials with saturated fat

solvents as adulterants. This table gives the results of each of ten successive measures of sulfuric acid containing 2 per cent of a saturated solution of butter fat in each of the various solvents. It will be seen that in the case of benzine, gasoline and xylol all ten tests checked within the customary limits. The slight variations may be accounted for by variations in the amounts of acid used, as the ordinary method of measuring acid is not accurate. In the case of ether and butterfat the successive samples did not check and after the third portion of adulterated acid there was no apparent effect upon the fat reading.

It will be noted from table 3 that the same percentage addition, to the acid, of saturated solutions of the different fat solvents did not give the same increase in fat reading. A 2 per cent addition of a saturated solution of fat in benzine gave an increased reading of 1.4 per cent while the same amount of a saturated solution of fat in gasoline and fat in xylol gave an increased reading of 1.2 and 0.7 per cent respectively.

Saturated solutions of other substances of group I in gasoline, benzine, xylol and ligroin increased the fat reading when added to the acid but so effected the color and consistency of the acid and the color of the fat column of the completed test that presence would be detected at once. For this reason detailed discussion of their behavior is omitted from here.

Detecting the presence in acid. Saturated solutions of butterfat in benzine, gasoline, and ligroin do not materially effect the appearance of the acid when added in moderate quantities. The fat columns of the completed test where such adulterated acid is used appears normal and duplicate samples of milk tested check within the permitted limits of variation. As a matter of fact there is nothing about the appearance of the acid or its behavior that will arouse suspicion. The only sure check being a blank test. A blank test on sulfuric acid containing 2 per cent of a saturated solution of butterfat in gasoline gave a reading of 1.1 per cent.

To get uniform results with successive samples of adulterated acid it was found necessary that the solvent must be saturated with butterfat. With gasoline it was found that if less than

15 grams of fat was added to 50 cc. of gasoline, part of the gasoline would rise to the top of the acid immediately after being shaken. In drawing off the acid, therefore, the first sample would contain a larger portion of gasoline than any of the succeeding samples and would give a larger fat reading.

It was also found that if oxidized butterfat was used in saturating the solvents there was less coloring of the acid.

V. SUMMARY AND CONCLUSIONS

1. Most fats and oils added to the sulfuric acid will increase the test of milk when such acid is used. All such fats and oils float on top of the acid and cause it to become very dark and sirupy, but do not effect its activity.

2. Many fat solvents when added to sulfuric acid will increase the test of milk where such acid is used. All float on top of the acid, but many do not effect the appearance of the acid nor its activity. Duplicate samples of milk where such acid is used will fail to check within permissible limits.

3. Saturated solutions of butterfat in gasoline, benzine and xylol will not materially affect the appearance of cold acid and will remain in solution with the acid for an hour or more after being thoroughly mixed. Successive samples of acid drawn will produce identical results; all samples checking in duplicate within the permissible limits of variation.

4. The presence of any substance in the sulfuric acid that will increase the test of milk when such acid is used in testing can be detected by making a "blank" test of the acid.

THE INFLUENCE OF PASTEURIZATION AND DIET OF THE COW ON THE ANTI-SCORBUTIC POTENCY OF THE MILK

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The vitamin content of milk apparently is influenced by two factors: viz., the diet of the cow, and the method or methods of preparing the milk for consumption. Kennedy and Dutcher (1) have reported that the presence of vitamins A and B in milk is entirely dependent upon the occurrence of these vitamins in the ration. This fact was substantiated by Osborne and Mendel (2) who observed no difference in the vitamin B content of milk produced on winter feed. Likewise Hughes, Fitch and Cave (3) fed cows a diet low in vitamins and observed that the A and B vitamin content of the milk to be very much decreased. The vitamin C content of milk appears to be more variable. Its presence in cows' milk is dependent upon the occurrence of the vitamin in the feed as well as the condition of the feed, and the manner in which it is cured.

Hart, et al. (4), of Wisconsin found that milk from cows which had been maintained on dried roughages and grains was lower in the anti-scorbutic vitamin than milk from cows on pasture. The following conclusions were deduced from this work:

Fifteen cubic centimeters dry feed-milk protected about six weeks.

Thirty cubic centimeters dry feed-milk protected about seven to eight weeks.

Fifty cubic centimeters dry feed-milk protected about twelve to fourteen weeks.

Seventy-five cubic centimeters of dry feed-milk gave complete protection over a period of eighteen weeks.

These data seemed to indicate that milk from dry-fed cows is deficient in vitamin C, but not wholly devoid of it. Hart

and Steenbock further concluded that the addition of silage and sugar mangels to the diet of the cow, did not greatly enhance the anti-scorbutic factor in milk. They report that the silage fed was made from corn well matured and partly dried before ensiling, a fact which might influence the anti-scorbutic value. The work of Hess, Dutcher, Kennedy and Unger seems to corroborate the work of Hart, et al., at the Wisconsin Station. All work on the various vitamins indicate that vitamin C is less stable in food products and is more easily destroyed by the various processes in the preparation of foods. This is no less true of milk, which is prepared for market.

There seems to be some differences of opinion among experimentalists as to the effects of pasteurization of milk in relation to its vitamin C content. Our work seems to point to the fact that there is a diminution of vitamin C, under all methods of pasteurization. The results obtained by Dutcher, et al., seem to indicate a greater destruction of vitamin C, when pasteurization is done in open vat or where oxidation can take place.

EXPERIMENTAL PROCEDURE

This experimental work was undertaken to determine the anti-scorbutic value of different amounts of milk when produced and treated under the following conditions: (*a*) Milk from cows on winter ration receiving corn silage compared with (*b*) milk from cows on winter ration, receiving no silage; (*c*) milk pasteurized in the bottle (final package) by the holding process 145°F., for thirty minutes, compared to fresh raw milk from the college herd.

The outline of purposes assumed that there might be differences in the anti-scorbutic properties of milk produced by cows on winter ration with and without silage. Also that the quality of the feed, including the condition and the method of curing are factors to be considered. The purposes further assumed that the methods used in pasteurization may also influence the vitamin content of milk.

Sixteen guinea-pigs were used for the work. Later 8 more were added. The pigs were young at the beginning of the trial

and weighed on an average of 300 grams. Before beginning the trial all the pigs were fed a complete diet. The pigs were weighed regularly, so as to detect any abnormal ones in the various lots. Each lot had about the same number of males and females and uniform in other respects. The pigs were kept in a cage with screen wire on both front and rear, each pig having a separate compartment about 12 inches square. Dry clean sawdust was used for bedding. The usual laboratory precautions were taken in regard to the sanitary conditions of the cages and dishes used for feeding. The milk was fed in aluminum dishes and were set in wooden blocks to prevent spilling and the pig allowed to drink milk ad libitum. The basal ration consisted of rolled oats, good quality autoclaved alfalfa hay cut fine, water, and salt. The alfalfa hay was autoclaved at 15 pounds pressure for thirty minutes and then dried.

Four pigs were placed on the basal ration; four more on basal ration plus pasteurized milk obtained from the College creamery. The milk was pasteurized in the bottle at 142° to 145°F. for thirty minutes and then cooled rapidly. Two pigs received 30 cc. daily, 1 pig 45 cc. daily and 1 pig 60 cc. daily of the pasteurized milk. In all cases the milk was fed in the dishes in two feeds, morning and evening. Four pigs were fed raw milk from the college herd plus the basal ration in the following amounts: 2 pigs received 30 cc., 1 pig received 45 cc., and 1 pig 60 cc. Later 4 more pigs were added to the experiment and fed the following amounts of milk from the college herd: 2 pigs received 15 cc. daily and 2 received 20 cc. daily plus the basal ration.

The college herd consists of the four principal dairy breeds with the Holsteins predominating. The herd was fed a grain ration of 4 parts of oats, 4 parts of corn, 2 parts of bran, and 1 of oil meal. During the early part of the winter and up to about February a very good quality of alfalfa hay was fed. The hay which was fed later in the year was not of as good quality. It was mixed with sweet clover, blue grass, and other tame grasses. The cows received all the corn silage they would eat. The silage was made from corn cut fairly green and was of very good quality.

Eight pigs were fed corresponding amounts of milk from a farmer's herd and the same basal ration. The herd from which this milk was obtained consisted of high grade Jerseys. Their grain ration consisted of ear corn and oats. For roughage they received an excellent quality of alfalfa hay up to the first part of February. This hay was very green and cured in cocks without caps. After the first week in February red clover hay was fed until April. From April until pasture season came on, a rather poor quality of alfalfa hay was fed. This hay was bleached and rather coarse also lacking in leaves. No succulent roughage of any kind was fed to the herd.

Composite samples of milk were taken over a period of several weeks and tested for butterfat.

Milk from college herd

	per cent
Composite sample 1	4.0
Composite sample 2	3.3
Composite sample 3	3.6
Composite sample 4	3.4
Composite sample 5	4.0
Composite sample 6	3.6
Average	3.65

Milk from farmer's herd

	per cent
Composite sample 1	5.1
Composite sample 2	5.4
Composite sample 3	5.5
Composite sample 4	5.0
Composite sample 5	5.1
Composite sample 6	5.3
Average	5.23

A complete analysis of the milk from the college herd was as follows:

	per cent
Total solids	12.61
Fat	3.7
Total protein	3.3
Lactose	5.0
Ash	0.74

The milk from the farmer's herd showed the following analysis:

	per cent
Total solids	14 2
Fat	5.3
Total protein	4.0
Lactose	4.2
Ash	0.75

The milk from both herds at all times appeared normal.

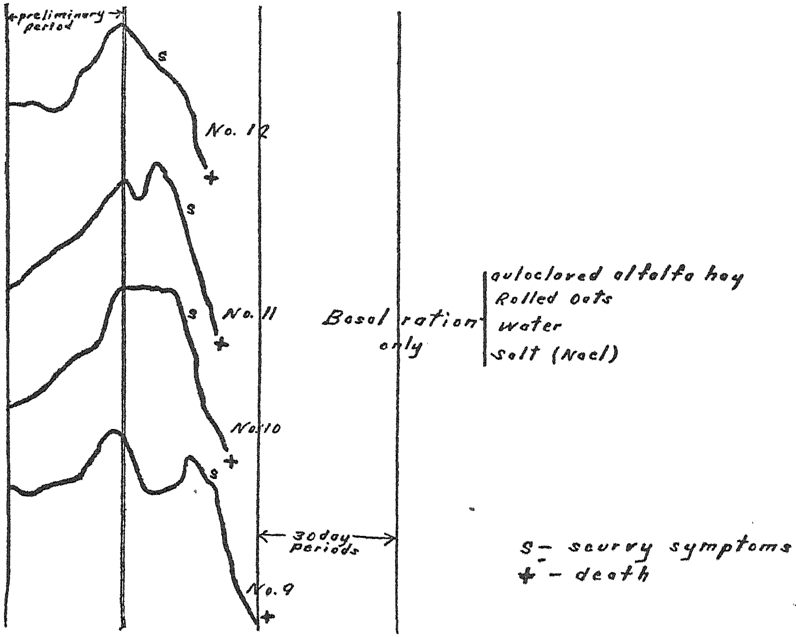

CHART 1

CARE AND MANAGEMENT

All animals were fed twice daily. A graduated pipette was used to measure the milk. All the milk was consumed before the following feeding period, except in several cases just prior to death. In no case was the milk force fed. The pigs were weighed every third day on a gram scale. All animals were autopsied as soon as possible after death. This work was done by a qualified veterinarian when the symptoms were not readily detected.

RESULTS

Chart 1 indicates clearly that the basal ration used was free from the anti-scorbutic vitamin. The rapid and uniform growth during the preliminary period is indicative of the good condition of the pigs before limiting their diet to the basal ration only. It is of interest to note also that all the pigs on the basal ration showed symptoms of scurvy about the same time, and 2 of the 4 died on the nineteenth day, the other 2 dying on the twenty-eighth and thirtieth days after being placed on the basal ration.

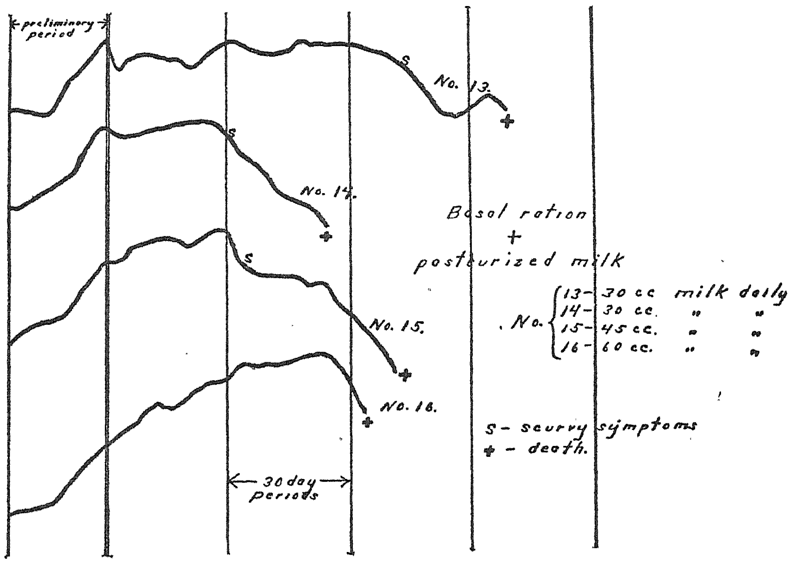

CHART 2

All the pigs were posted immediately after death, and the following conditions were noted: The costochondrel junctions were enlarged and inflamed. There were marked hemorrhagic areas in the thighs. The kidneys were also more or less hemorrhagic. Brittleness of the bones, particularly the leg bones was very noticeable. The contents of the cecum and intestines were soft and pasty; no cases of impaction was noted, on the contrary pig 9 had diarrhea for several days previous to death.

BASAL RATION AND PASTEURIZED MILK

The results of the trial with pasteurized milk in addition to the basal ration were variable. Pig 14 in chart 2 lived fifty-four days on 30 cc. of this milk daily, while pig 13 receiving the same amount of pasteurized milk did not die until the ninety-seventh day. Although making no gain in weight, pig 13 showed unusual resistance. Pig 15 receiving 45 cc. of pasteurized milk daily lived seventy-two days. Pig 16 on 60 cc. of pasteurized milk died on the sixty-fifth day. On post-mortem, scurvy symptoms were evident; however, the immediate cause of death in case of

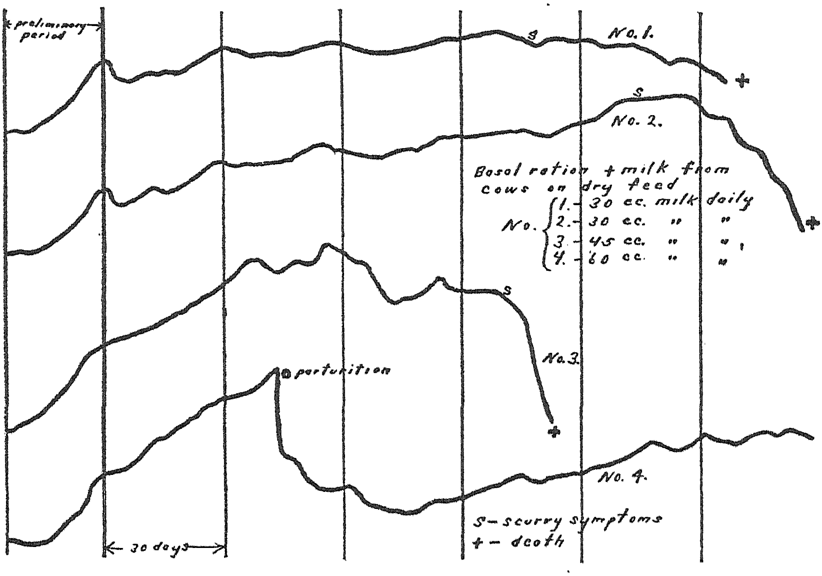

CHART 3

this pig was attributed to penumonia. It is possible that the lowered vitality due to the ration was a factor in bringing on pneumonia as this was the only case in which death could be attributed to any cause other than a deficiency ration.

BASAL RATION AND MILK FROM FARMER'S HERD

Pigs 1, 2, 3 and 4 on chart 3 were fed milk from cows on dry feed only for the duration of the experiment of twenty-five

weeks. Three of these pigs (nos. 1, 2 and 3) developed scurvy and died. Pig 3 died on the one hundred and eighth day. Death was probably hastened by loss of all four incisor teeth. The teeth became brittle and broke off at the gums. This made it difficult for the pig to eat solid foods. Pig 2 died a few days previous to the conclusion of the trial. Pig 4 was pregnant when the trial started, and after forty-two days on the restricted diet she gave birth to 1 young. The young pig showed no

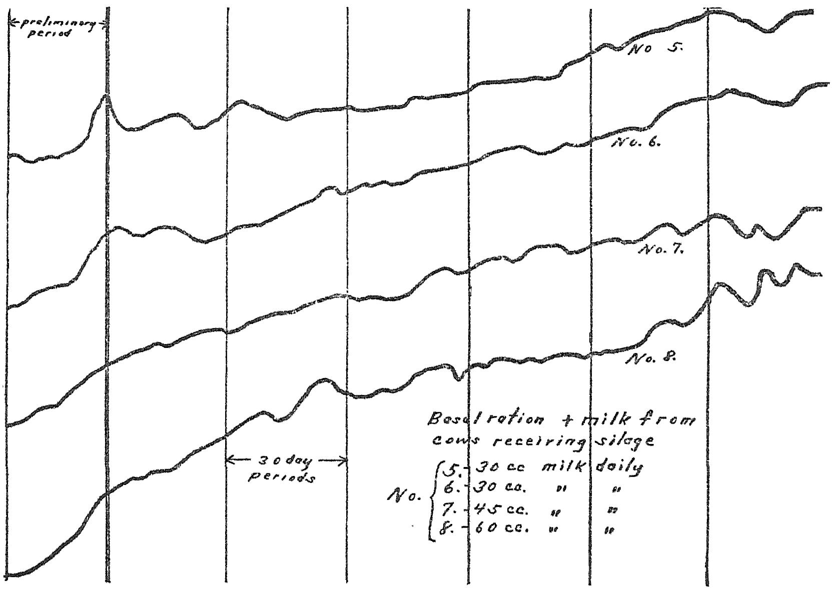

CHART 4

scurvy symptoms when born and was allowed to suckle for two weeks. Shortly after her parturition, pig 4 fell and injured her back. She recovered from these incidents and made good growth during the last sixty days of the trials.

BASAL RATION AND MILK FROM COLLEGE HERD

Chart 4 which shows the growth of pigs receiving milk from the college herd is a marked contrast to chart 3. The pigs shown on the latter chart received the same amount of milk

and the same basal ration. At the end of the trial all of these pigs were in a vigorous, healthy condition, with a glossy coat. Three of these pigs, no. 6 on 30 cc. of milk, no. 7 on 45 cc. of milk and no. 8 on 60 cc. of milk, doubled their initial weight during the trial.

Chart 5 shows the growth curve of two other lots of 4 pigs each, which were started on the trial February 28. Pigs 13′, 14′, 15′, 16′ received in addition to the basal ration the following

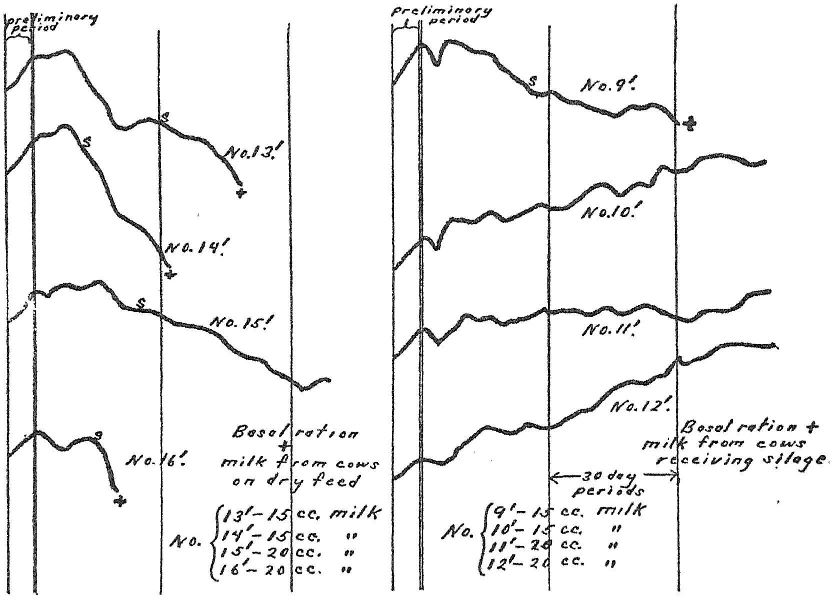

CHART 5

amounts of milk from the farmer's herd. Pigs 13′, and 14′, received 15 cc. of milk daily. Pigs 15′, and 16′, received 20 cc. of milk daily. All 4 of the pigs on milk from the farmer's herd developed marked symptoms of scurvy in less than thirty days. Two died within that time. Pig 13′ died on the forty-eighth day and pig 15′ did not die before the termination of the trial. Pigs 9′, 10′, 11′, 12′ were fed similar amounts of milk from the college herd. Pig 9′ was the only one which showed scurvy symptoms. (This pig died on the sixty-third day after the

trial began.) The other 3 pigs showed good growth, and their gains in weight were as good as could be expected on such small quantities of milk. The data in this write up comprises a period from November 4 to June 1 of the following year. The pigs which were still alive after this date were continued up to July 1 with results very similar to those already recorded. But inasmuch as both herds were then receiving more or less pasture which would involve another factor it was thought best to omit this data. It is of interest to record that no noticeable effect was observed on the pigs receiving milk from the farmer's herd, even after the herd had been on pasture for some time. However, the condition of the pigs at this stage of the trial no doubt was an important factor. A second trial involving the same factors was started last October. The results are essentially the same as those recorded herein. A more complete analysis of the three trials conducted at this institution on vitamin C in milk will appear in print later.

CONCLUSIONS

1. The results obtained from the pigs on the basal ration indicate that the ration used was free of vitamin C.
2. Pasteurization in closed bottles at 142°F., held at that temperature for 30 minutes diminishes the vitamin C content of milk.
3. These data indicate that the vitamin C present in milk has its origin in the feed.
4. Silage made from corn, cut when the kernels are glazed but before the lower leaves had dried increased considerably the anti-scorbutic potency of milk from cows receiving such silage.
5. Good silage contains sufficient vitamin C to maintain an adequate supply of this vitamin in the milk.

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A COLORIMETRIC PICRIC ACID METHOD FOR DETERMINING LACTOSE

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In this paper we describe a picric acid colorimetric method for the determination of lactose in milk and some of its products. The results obtained by this method are compared with those obtained using the Official Gravimetric Fehling Method—Munson and Walker procedure.

REVIEW OF LITERATURE

The picric acid colorimetric method, for the estimation of carbohydrates, owes its origin to Dehn and Hartman (1) who worked with purified sugars and lactose in milk; and to Lewis and Benedict (2) who worked on glucose in blood. The method is based upon the fact that reducing sugars, in alkaline solution, (when heated) reduce picric acid which is yellow in color, to picramic acid which is a deep mahogany red. The original methods have been improved and modified for application to various sugar containing materials. Several different modifications, as applied to milk, have been published. Folin and Denis (3), report a modification using saturated picric acid and a standard which must have a color value within 20 per cent of the color value of the unknown. Pacini and Russell (4) use more complicated technique and solid picric acid with no restriction on the comparative colors of the standard and unknown. Boch (5), reports a study on the reaction of lactose on picric acid using Benedict's (6), "three times saturated solution," which is in reality sodium picrate. The method of Folin and Denis was used by Lisk (7) in her work on milk. To our knowledge, no one has applied any picric acid method to dairy products, other than milk.

EXPERIMENTAL

In the method described we have adopted the procedure of Folin and Denis for removing the protein and fat; but have used different dilutions and concentrations in the determination proper. A correction table has also been worked out to overcome the necessity of having more than one standard solution. Furthermore the method has been extended to cover some other dairy products.

In our preliminary work we found that pure lactose solutions and milk gave low results when they contained less lactose than the standard and high results when they contained more lactose than the standard, when calculated by the usual proportion method. This is due to the fact that the color developed is not proportional to the amount of lactose (or other sugars) present unless a much higher concentration of picric acid or sodium picrate is used (with greater complexity of technique) than under our procedure. It is for this reason that Folin and Denis require the colors of the unknown and the standard to the within 20 per cent of each other, thereby partially overcoming the error; and other modifications recommend sodium picrate (which is more soluble), solid picric acid, evaporation to dryness, etc., in their respective procedures. In the method reported here we have used a saturated solution of picric acid and overcome the error, otherwise present, by using a table of factors, which we have worked out. This table not only makes the procedure more simple but shortens the calculation of results somewhat.

The table of factors is based on 118 determinations made on 58 different lactose solutions. The difference in concentration of the lactose solutions varied from 300 to 700 mgm. in 500 cc. distilled water with 10 mgm. difference between individual solutions. This would be equivalent to from 3 to 7 per cent of lactose on the basis of milk. Color values representing these lactose solutions were obtained by the procedure described in this paper, using a 0.1 per cent water solution of lactose as the standard. The standard color was set at 20 on a Kober colori-

TABLE 1

Factors

A. Colorimeter reading of unknown compared with standard at 20.
B. Factor to use in calculation of results.

A	B	A	B	A	B	A	B	A	B
13.0	1.465	17.0	1.164	21.0	0.959	25.0	0.832	29.0	0.733
13.1	1.455	17.1	1.158	21.1	0.956	25.1	0.829	29.1	0.731
13.2	1.446	17.2	1.152	21.2	0.952	25.2	0.826	29.2	0.728
13.3	1.437	17.3	1.146	21.3	0.948	25.3	0.823	29.3	0.726
13.4	1.428	17.4	1.139	21.4	0.945	25.4	0.821	29.4	0.724
13.5	1.419	17.5	1.133	21.5	0.941	25.5	0.818	29.5	0.722
13.6	1.410	17.6	1.127	21.6	0.937	25.6	0.815	29.6	0.720
13.7	1.401	17.7	1.121	21.7	0.934	25.7	0.812	29.7	0.718
13.8	1.392	17.8	1.115	21.8	0.930	25.8	0.810	29.8	0.716
13.9	1.384	17.9	1.109	21.9	0.927	25.9	0.807	21.9	0.715
14.0	1.375	18.0	1.103	22.0	0.924	26.0	0.805	30.0	0.713
14.1	1.367	18.1	1.097	22.1	0.921	26.1	0.802	30.1	0.712
14.2	1.359	18.2	1.091	22.2	0.917	26.2	0.800	30.2	0.710
14.3	1.351	18.3	1.085	22.3	0.914	26.3	0.797	30.3	0.708
14.4	1.343	18.4	1.079	22.4	0.910	26.4	0.795	30.4	0.706
14.5	1.335	18.5	1.073	22.5	0.907	26.5	0.792	30.5	0.704
14.6	1.328	18.6	1.067	22.6	0.904	26.6	0.790	30.6	0.702
14.7	1.320	18.7	1.062	22.7	0.901	26.7	0.787	30.7	0.700
14.8	1.312	18.8	1.056	22.8	0.898	26.8	0.785	30.8	0.698
14.9	1.305	18.9	1.051	22.9	0.895	26.9	0.782	30.9	0.696
15.0	1.298	19.0	1.045	23.0	0.892	27.0	0.780	31.0	0.694
15.1	1.291	19.1	1.040	23.1	0.889	27.1	0.777	31.1	0.693
15.2	1.284	19.2	1.035	23.2	0.886	27.2	0.775	31.2	0.691
15.3	1.277	19.3	1.030	23.3	0.883	27.3	0.773	31.3	0.689
15.4	1.270	19.4	1.025	23.4	0.880	27.4	0.771	31.4	0.687
15.5	1.263	19.5	1.021	23.5	0.877	27.5	0.768	31.5	0.686
15.6	1.256	19.6	1.016	23.6	0.874	27.6	0.766	31.6	0.684
15.7	1.249	19.7	1.012	23.7	0.871	27.7	0.764	31.7	0.683
15.8	1.242	19.8	1.008	23.8	0.868	27.8	0.762	31.8	0.681
15.9	1.235	19.9	1.004	23.9	0.865	27.9	0.759	31.9	0.679
16.0	1.229	20.0	1.000	24.0	0.862	28.0	0.757	32.0	0.677
16.1	1.222	20.1	0.995	24.1	0.858	28.1	0.755	33.0	0.662
16.2	1.215	20.2	0.991	24.2	0.855	28.2	0.752	34.0	0.647
16.3	1.208	20.3	0.986	24.3	0.852	28.3	0.750	35.0	0.633
16.4	1.201	20.4	0.982	24.4	0.849	28.4	0.748	36.0	0.619
16.5	1.195	20.5	0.978	24.5	0.846	28.5	0.746	37.0	0.606
16.6	1.189	20.6	0.974	24.6	0.843	28.6	0.744	38.0	0.594
16.7	1.183	20.7	0.970	24.7	0.840	28.7	0.741	39.0	0.582
16.8	1.176	20.8	0.966	24.8	0.837	28.8	0.738	40.0	0.571
16.9	1.170	20.9	0.963	24.9	0.834	28.9	0.735		

meter. The lactose used was tested for purity by the Official Gravimetric Fehling Method (Procedure according to Munson and Walker) and a slight correction made before the standard was made up. A curve was then plotted—the abscissas being the concentration of lactose and the ordinates the colorimeter readings. It was then a simple matter to tabulate the lactose values of solutions corresponding to different colorimeter readings. This table constitutes an automatic correction and is a

TABLE 2

Recovery of lactose in solutions of known strength using table of factors

SOLUTION NUMBER	GRAMS WEIGHED OUT (PER 100 CC.)	GRAMS OBTAINED BY ANALYSIS (PER 100 CC.)	PER CENT ON BASIS OF MILK		VARIATION B–A
			Weighed out A	By analysis B	
1	0.0700	0.0696	3.50	3.48	−0.02
2	0.0842	0.0843	4.21	4.21	0.00
3	0.0932	0.0941	4.66	4.70	+0.04
4	0.1058	0.1050	5.29	5.25	−0.04
5	0.1219	0.1226	6.09	6.13	+0.04
6	0.1303	0.1366	6.91	6.83	−0.08
7	0.1331	0.1320	6.65	6.60	−0.05
8	0.1033	0.1008	5.11	5.04	−0.07
9	0.1117	0.1099	5.57	5.50	−0.07
10	0.1160	0.1164	5.80	5.82	+0.02
11	0.0659	0.0656	3.27	3.28	+0.01
12	0.0839	0.0844	4.19	4.22	+0.03

necessary adjunct to our procedure. Table 1 gives the factors with their corresponding colorimeter readings.

The accuracy of the table of factors was tested by determining the lactose in water solutions of known strength. Table 2 gives the results of this check. Pure lactose was weighed out, made up to 500 cc. and then recovered by analysis in terms of grams of lactose per 100 cc. and percentage on the basis of milk.

The results show that the accuracy of the method on the basis of milk is well within 0.1 per cent, the average difference being 0.04 per cent and the maximum difference noted on these solutions 0.08 per cent.

In our work thus far, several interesting and important ob-

servations were made. It was found that by filtering a portion of the solutions after the development of the color, higher colorimeter readings (lower colors) were obtained than on the unfiltered portions, showing that filtering results in a loss of color. By filtering the standard also it was possible to obtain the initial reading again. Because of this fact, if for any reason the unknown solution is filtered, after color development, the standard color solution must also be filtered to obtain accurate results.

Another point of importance, effecting results, unless procedure is accurately followed, is that after removing the solutions from the boiling water bath, the colors gradually fade for a period of time. For this reason it was found necessary to develop the standard color simultaneously with the unknown. This is also necessary because variations in length of heating periods, vigorousness of boiling, etc., give rise to variations in color. It therefore follows that a permanent color standard, except for approximate results, is not advisable; but if used, the time of heating and interval between heating and reading should be accurately controlled.

We attempted several methods of preserving the standard water solution of lactose for the use over a period of time. Formaldehyde gave a higher color when present in appreciable quantities and mercuric chloride on the other hand gave a lower color. It was therefore impossible to preserve the standard with either of these preservatives. We next tried making up the lactose solution in saturated picric acid. This method seems to give very good results. A standard was kept in saturated picric acid for over two months and the color developed at the end of that time was within experimental error of the color developed when freshly made. Either lactose is not inverted in appreciable quantities in a cold solution of picric acid, or what is more probable, the reducing powers of lactose, and the invert sugar from lactose, are the same on picric acid. We have made no thorough investigation of this point but some of our results would seem to indicate that even though inversion does take place, the final amount of picramic acid produced is the

same or so close that no difference is perceptible in the colorimeter. A standard solution in saturated picric acid was incubated at about 40°C. for five days, without any appreciable change in its final color value. We are, therefore, of the opinion that such a standard can be made up and kept for six or seven weeks for analytical purposes, without the necessity of preparing water solutions every day or so. This method of preparing a standard solution would be of value only where analyses are to be made daily or every few days, over a period of time. In using this type of standard solution it is advisable to use the same saturated solution of picric acid that is to be used in the subsequent determinations, since the amount of picric acid in solution should be the same and this is not always easy to be sure of.

METHOD AND PROCEDURE

Reagents

Picric acid. A saturated solution of picric acid in distilled water is used. Picric acid goes into solution extremely slow in the cold so a supersaturated solution is prepared by heating which is allowed to crystallize out at room temperature before use.

Sodium carbonate. Pure, anhydrous sodium carbonate is dissolved in distilled water at the rate of 22 grams per 100 cc. Heating facilitates the solution of the sodium carbonate.

Standard lactose solution. Exactly 1 gram of pure dry lactose monohydrate is dissolved in 1 liter of distilled water (or proportionate amounts in other volumes). For exacting work, a determination should be made on the lactose as a check on its purity, before making up the standard.

Note: As set forth in the description of experimental work, a standard may be made up in saturated picric acid and used for six or seven weeks with safety.

Preparation of standard color

Transfer 10 cc. of the standard lactose solution to a 100 cc. volumetric flask add 20 cc. of saturated picric acid (10 cc. when

the standard is made up in saturated picric, plus 10 cc. of water) and 10 cc. of 22 per cent sodium carbonate. Mix, cork lightly and heat simultaneously with unknowns in a boiling water bath for fifteen to twenty-five minutes; cool in cold water, simultaneously with unknowns, and dilute to 100 cc. Transfer a portion of the standard color solution to the colorimeter cup and place on left side of colorimeter, setting it at 20. The table of factors is based on the standard color being set at 20. (If set at any other point the reading of the unknown must be calculated to the value it would have if the standard was at 20, before the table of factors can be used.) The unknown solutions, representing the lactose content of the various samples, are compared with the standard color and readings taken. Due to the slight fading in color, it is advisable to change the standard color in the cup with each unknown and reset at 20. It is necessary to prepare a new standard color with each set of determinations and it is absolutely necessary to prepare the standard color along with the unknown under identical conditions for accurate results.

Milk

The specific gravity is determined with a Westphal balance or an accurate lactometer. With an accurate 2 cc. pipette, transfer 2 cc. of milk to a 100 cc. volumetric flask, previously half-filled with saturated picric acid solution. The pipette used should be one that will deliver exactly 2 cc. of milk. Fill to the mark with saturated picric acid, shake and filter. Transfer 10 cc. of the filtrate to another 100 cc. volumetric flask; add 10 cc. distilled water, 10 cc. saturated picric acid and 10 cc. of 22 per cent sodium carbonate. Mix, cork lightly and heat simultaneously with standard lactose solution, in a boiling water bath for fifteen to twenty-five minutes. Cool in cold water simultaneously with the standard color; dilute to 100 cc. and mix. Transfer a portion of the colored solution to the colorimeter cup and place in the right side of the colorimeter. The color is matched with the standard color in the usual manner. The average of five readings constitutes the color value.

Calculation. The percentage of lactose is found by obtaining the factor corresponding to the color value of the unknown solution from table 1 and using either of the following formulas.

$$\frac{\text{Factor} \times 10}{\text{Weight of sample}} = \text{per cent lactose}$$

or

$$\frac{\text{Factor} \times 5}{\text{Specific gravity of milk}} = \text{per cent lactose}$$

Cream

In determining the lactose content of cream, approximately 2 grams of cream are weighed into a 100 cc. volumetric flask previously half filled with saturated picric acid, using a Mojonnier weighing cross and pipettes. Fill to the mark with saturated picric acid, shake and filter. The subsequent procedure and calculation is then the same as for milk.

Whey

Approximately 2 grams or exactly 2 cc. of known specific gravity are used, with procedure similar to milk.

Evaporated milk

Approximately 1 gram of evaporated milk is weighed into a 100 cc. flask as for cream and the same subsequent procedure followed.

Powdered milk

In powdered milk we have a product which is often lumpy and it is advisable to mortar all samples before weighing out a representative portion. This applies especially to whole milk powders.

Weigh quickly, about 0.2 gram of the fine powder on a small watch glass. Transfer to a 100 cc. flask with the aid of a small funnel, washing the powder in with saturated picric acid. Shake vigorously before making to the mark or until no lumps of powder

are noticeable. Make up to the mark with saturated picric acid, mix and filter, proceeding as under aforementioned products.

Because of the small size of the sample and the large dilution. this method is not accurate to within less than 0.3 or 0.4 per cent of lactose in the powder. Other methods, however, have to contend with more or less the same difficulty depending on the amount of diluton used.

Ice cream

In ice cream we have a product, which not only contains lactose, but has considerable quantities of added sucrose as well. We have found by experimentation that the sucrose does not interfere with the determination of lactose by our procedure, providing that the sucrose does not become hydrolized during the time the product is in picric acid solution. Sucrose will invert comparatively quickly in the presence of saturated picric acid in the cold, and produce high results, whereas uninverted it gives no reduction.

In the determination approximately 1.8 grams of melted ice cream or ice cream mix, are weighed into a 100 cc. volumetric flask by means of a Mojonnier weighing cross and pipettes. The flask is then quickly made up to the mark with saturated picric acid, well shaken, and filtered. The first 10 cc. of clear filtrate are then rapidly transferred to another 100 cc. flask and the usual procedure followed. It is advisable to get the sodium carbonate into the flask within fifteen minutes of the time the sample is first introduced into the saturated picric acid, otherwise high results will be obtained due to inversion of some of the sucrose.

Condensed milk

Condensed milk has more sucrose present than does ice cream so that precautions in regard to inversion are even more important in analyzing it. The procedure is entirely the same as for ice cream except that only 1 gram of sample is used. It is

TABLE 3

Comparison of lactose content of various products by the colorimetric and gravimetric methods

PRODUCT	SAMPLE NUMBER	COLORIMETRIC METHOD (A)	FEHLINGS METHOD (B)	VARIATION A–B	COLORIMETRIC METHOD (1 cc.)
		per cent	*per cent*	*per cent*	*per cent*
Milk	1	4.75	4.82	−0.07	4.75
	2	4.70	4.76	−0.06	
	4	4.81	4.80	+0.01	4.75
	5	5.69	5.73	−0.04	5.74
	6	3.75	3.70	+0.05	3.81
	13	5.32	5.26	+0.06	5.31
	Average......................			0.05	
Cream	10	3.67	3.72	−0.05	3.68
	19	4.88	4.88	0.00	4.89
	20	3.71	3.72	−0.01	3.72
	Average......................			0.02	
Whey	16	4.65	4.65	0.00	4.70
	17	5.28	5.35	−0.07	5.22
	Average......................			0.04	
Evaporated milk	7	9.93	10.14	−0.21	
	9	7.86	7.72	+0.14	7.86
	18	9.59	9.45	+0.14	9.62
	24	9.34	9.30	+0.04	9.41
	Average......................			0.12	
Powdered milk	3	49.90	50.48	−0.58	50.05
	14	49.40	49.56	−0.16	49.75
	Average......................			0.37	
Ice cream	11	4.39	4.41	−0.02	
	12	3.87	3.96	−0.09	
	22	4.46	4.52	−0.06	4.45
	23	6.69	6.89	−0.20	6.64
	Average......................			0.09	
Condensed milk	8	10.08	9.80	+0.28	
	15	8.99	8.58	+0.41	
	21	12.35	12.45	−0.10	
	Average......................			0.26	

important to get a representative sample from the can or batch. Many cans have "sugar down" and representative portions are hard to obtain. In bad cases a primary dilution with warming, may be advisable. Such dilution should not be made with more than an equal volume of water, in which case 2 grams of the dilution may be used as the sample for analysis. It is advisable not to have any picric acid in the flask when the sample is weighed into it, since this will increase the time the sample will be in picric acid.

ACCURACY OF METHOD

In table 3 the results of several determinations by this picric method on the products listed under procedure are given, as are also the results on the same samples by the official gravimetric Fehling method. The amount of variation between results by the two methods is shown with the average for each product.

In the last column (headed "colorimetric method 1 cc.") we record results on some of the samples which were made up to 10 cc. instead of 100 cc. This was done by taking 1 cc. of the primary dilution (after filtering) instead of 10 cc. and using one-tenth the amount of reagents, the boiling being done in 10 cc. graduated test tubes and the tubes made up to 10 cc. just as the flasks are made up to 100 cc. The results by this variation in technique are fairly accurate and for many purposes this way of making the determination would doubtless suffice.

DISCUSSION

In view of the results obtained on the lactose content of milk and some of its products, the colorimetric picric acid method described, seems to be as accurate as the so-called "official methods," basing this assertion on a comparison with the Munson and Walker Fehling method. It has an advantage over the older methods in being quicker, less complicated, less tedious and in requiring fewer and more easily prepared reagents. The method is of special advantage where a large number of analyses are to be made at one time.

On fluid products, where a 2 gram sample is used, results are within experimental error, of the older method, averaging only about 0.04 per cent difference. On more concentrated products the variation in per cent is greater due to the greater dilution of sample that is necessary, or to the smaller sample taken. This however, is to be expected and most other methods have the same thing to contend with to a greater or lesser extent.

The main advantage of our modification over the method of Folin and Denis, we believe to be in the use of the table of factors which we have employed. This overcomes the error due to the fact that the color developed is not proportional to the amount of sugar present.

We expect to continue the work on this method and endeavor to apply it to determinations of sucrose as well as lactose in such products as ice cream and condensed milk.

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THE OFFICIAL TEST IN THE SOUTH AND THE PRELIMINARY MILKING[1]

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Over most of the South official testing was not undertaken as early as in the North. Some few of the Southern states, namely, Maryland, Kentucky and probably Virginia and Tennessee, did official testing from the first years. Since the arrival of the boll weevil the Southern farmer, who was formerly content with raising cotton, has taken up other lines of farming. A great many have become interested in dairying and this branch of farming has continued to attract attention at an increased degree with each succeeding year. Increased interest has created a larger demand for better cattle. This demand for improved cattle has induced an increased call for the Official Test each year. The growth of the Register of Merit of the Jersey Cattle Club in Mississippi well represents the advance of the Official Test in the South. Dairying was not followed we might say at all in Mississippi until 1909 or 1910 when the boll weevil commenced to make his work felt. In 1911 there were 3 cows entered in the Register of Merit from Mississippi; 1912, 3; 1913, 0; 1914, 0; 1915, 1; 1916, 3; and 1917, 4. In 1922, 36 cows were entered; an increase of 32 over 1917. The growth between these years was steady. This from one of the foremost cotton states well represents the growth of the dairy industry over a large section of the South. A large percentage of the breeders would be classed as small, and are testing 1, 2, or 3 cows at a time. It is a small man proposition.

Some of the things accomplished by the Official Test are:

1. Increased value of the animals completing the test.

[1] Read at the annual meeting of the Southern Division, American Dairy Science Association, Birmingham, Ala., January 10, 1924.

2. Increased value of the offspring of the animal tested. These two facts are substantiated by every sale where tested and untested animals are put up at auction.

3. An index as to the ability of the parents to produce animals with ability to produce and, in fact, to reproduce, or the value of the animals for breeding purposes.

4. Affects the mental attitude of the breeder himself, very many times because of his increased interest in his herd causing him to give it better care and feed.

The large breeder is better able to help himself than is the smaller breeder. He does not need outside aid and encouragement as does the smaller breeder. We believe that the small breeder is the one that should be encouraged to use the Official Test and to this end the test should be kept within his reach. The preliminary milking rule, if enforced will increase the cost of testing a cow on the average \$3 to \$4 a year. To the man with 1, 2, or 3 cows more will be added. Many breeders feel that they are paying the limit for testing now and to add an additional cost is the straw that will break the camel's back. Perhaps they are right. At least we should not add any additional expense until thoroughly proved necessary. Is the preliminary milking absolutely required to ensure a correct test? I wish to give some data collected at the Mississippi Station by the writer.

Our work is divided into five periods: normal, I, II, III, and IV.

Data collected in the spring of 1921 when tests were run on 8 cows for one month, during which the cows were under normal conditions and each milking was weighed and sampled and the sample tested for butterfat, was used to make up the Normal Period.

In the spring of 1923 we ran tests on the individual milkings of 2 Ayrshires, 2 Holsteins and 2 Jerseys for thirty days. These cows were in different stages of lactation, one advanced and one in the early stages being selected for each breed. They were milked twice a day. Three times during the thirty days milk averaging 36.6 per cent of the normal amount for that milking was left in the udder. This is known as period I.

Following this test, we ran similar tests on 5 Jerseys that were on Register of Merit work and were milked three times daily.

These cows were of different ages; 2 with their first calves, 2 with their third calves and 1 a cow over twelve years of age. All completed records that averaged over 500 pounds of fat. Two were in the eleventh and twelfth month of their lactation period and the other 3 in the third and the sixth. These cows were watched over a period of nearly seventy-five days. During the first two thirty-day periods, milk to the amount of 27 and 31 per cent, respectively, of the average amount for that milking was left in the udder at the last milking of the day. The last period of thirteen days was of normal milkings. These periods are periods II, III, and IV, respectively.

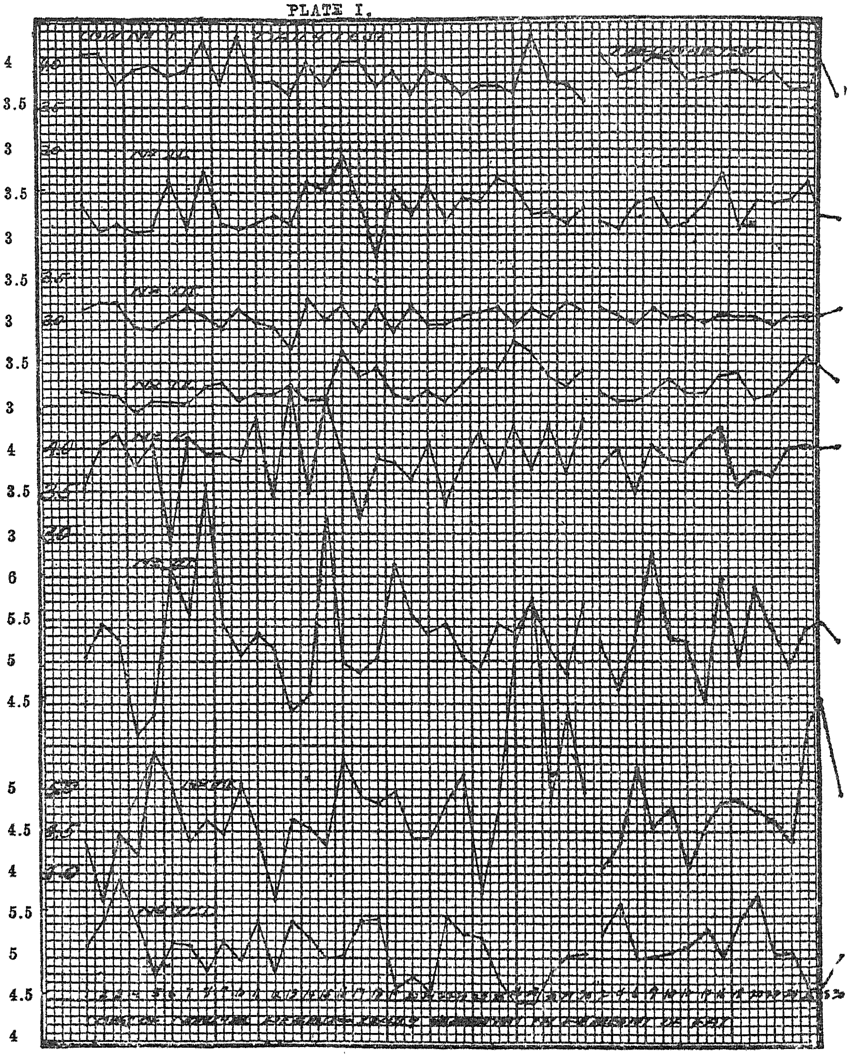
Does leaving the milk in a cow's udder influence the variation of the daily tests of her milk? In the following table the total variations of the tests of the milk of the individual cows of each group from one day to the next are collected and classified as to whether minus or plus and as to whether 0.4 per cent or less or over 0.8 per cent.

Daily variations of test

PERIOD	Total	VARIATION											
		0.4 per cent or less						Over 0.8 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	232	81	34.90	73	31.5	154	66.40	10	4.3	16	6.9	26	11.2
I	174	58	33.33	63	36.2	121	69.50	7	4.0	10	5.7	17	9.7
II	145	58	40.00	48	33.1	106	73.10	1	0.69	4	2.76	5	3.45
III	143	48	33.57	47	32.87	95	66.40	4	2.8	7	4.9	11	7.7
IV	48	21	43.75	11	22.9	32	66.65	2	4.2	0	0	2	4.2

Comparing the percentage variation of 0.4 per cent or less we find that the minus and the plus variations are about equal in all the periods. The greatest difference is in period IV when the cows were under normal conditions. The next greatest difference is in period II when the percentage of minus variations increases and this increase occurs at the expense of the

PLATE I.

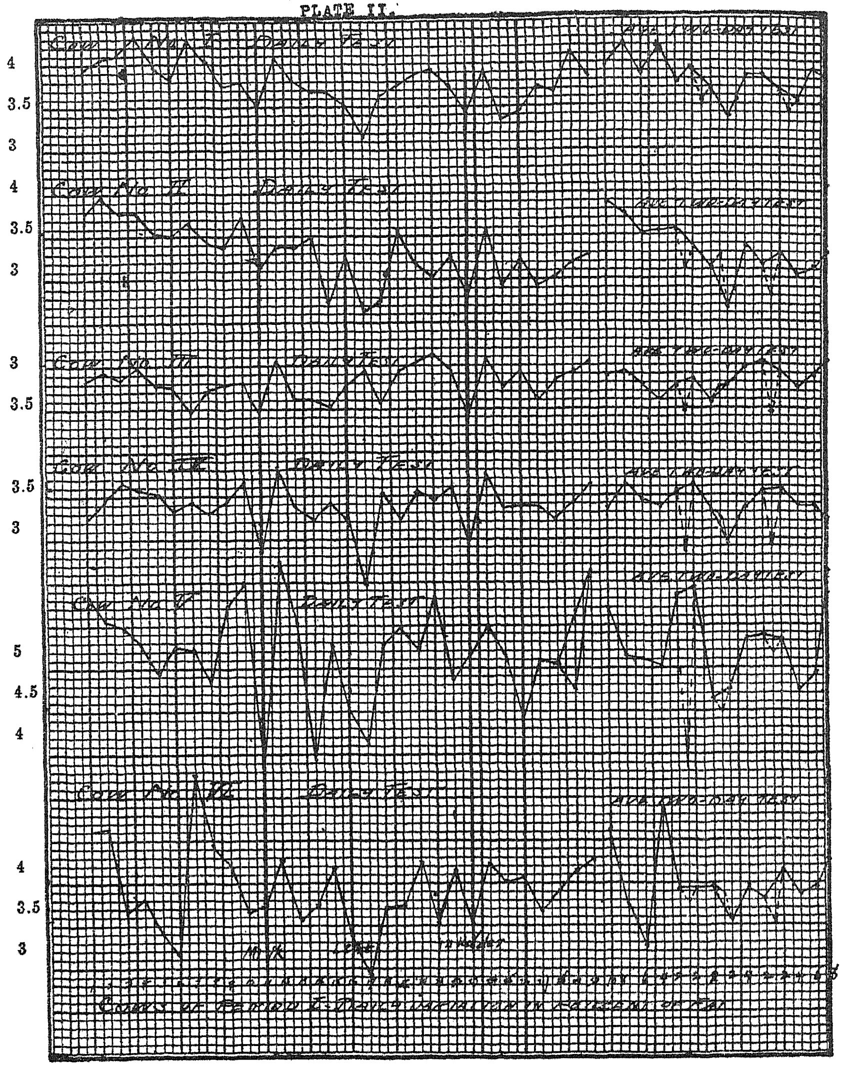


variations of greater than 0.8 per cent. The total variations of 0.4 per cent or less are more in the periods when the milking was interfered with, while the variations of more than 0.8 per cent are less. This study does not reveal any increased variation of the tests in the periods in which the milk was left in the udder.

We learn from this study that the tendency is for the amount of variation to increase as the average per cent of fat in the cow's milk increases and that the amount of variation varies with the individual cow. The test also seems to fluctuate from day to day about a certain medium point though this point may in the course of a month tend to either rise or decline. The plus deviations when totalled are always approximately equal to the sum of the minus deviations for that month. When there is a change in the test for a certain interval of time, usually one or two days, there is a corresponding change in the other direction, either following or preceding. This is very nicely brought out in the graph illustrating the variations of the test of the cows of the normal group as shown on figure 1. From this graph we learn that the least limit of variation is with the cow having the lowest average test while the greatest limit is with that cow having the highest average test. A study of the tests of the cows used during the other periods show that the limits of variation in the tests conform in a similar way to the average test and to the individual cow. This is illustrated on figures 2 and 3 with graphs showing the variations in test of the cows used in periods I and II.

A study of the daily tests of the individual cows used in our work does not give any evidence that leaving the milk in the udder at any one time has influenced the direction of the variation of the test, unless on the day when the milk was left in the udder when the tendency is for the test to be lowered. Consider cow II (fig. 3). At the time the milk was left in the udder first her test shows a downward tendency. This downward slant continues the two days following the day on which the milk was left in the udder. At the next time the milk was left in the udder she also had a downward tendency. Leaving the milk in the udder threw the test lower and the following day it came back to exactly where it was and then fell again. At the last interference with the normal milking of this cow in this period she was due for a rise her test being very low the day before. This rise occurred on the day the milk was left in the udder her test going from 5.207 to 6.327 and then fell to 6.025. This

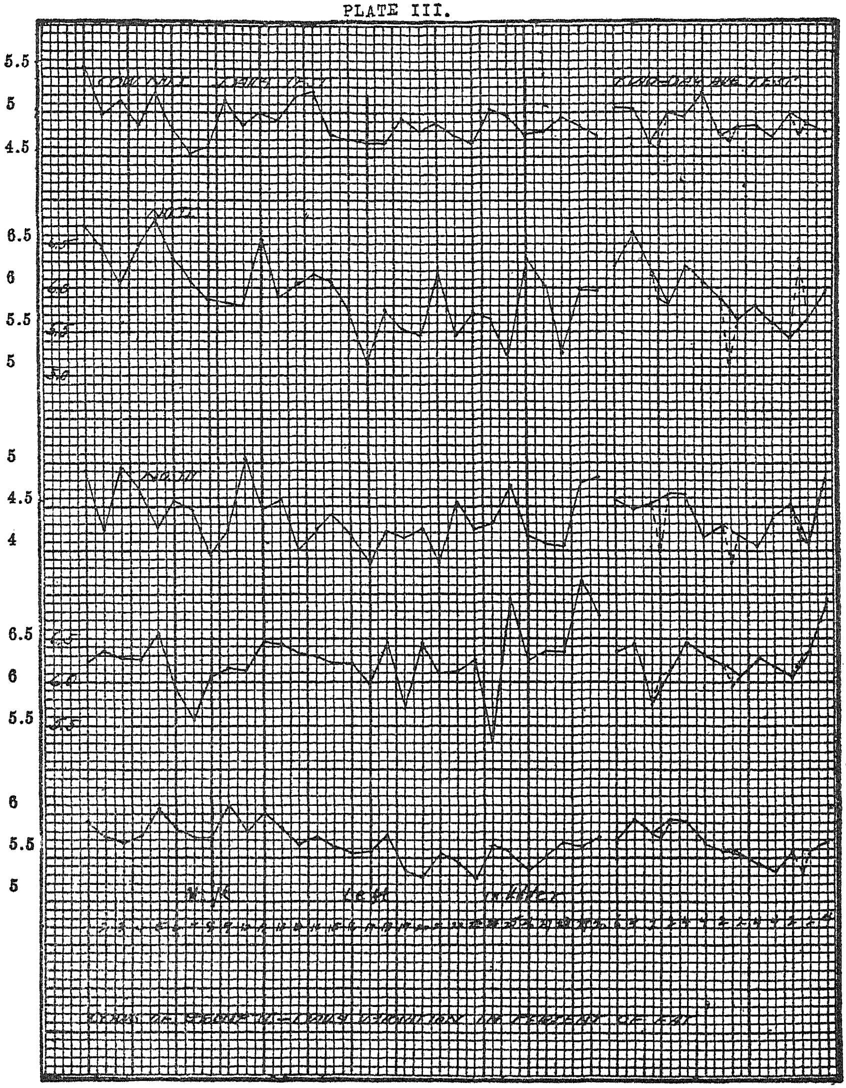
PLATE II.



decline continued the next day after which there was another rise in the test.

The Official Test is based on a two-day average test. Do the facts as given for the daily average hold for the two-day average?

PLATE III.



The following table shows the total variations of the average two-day tests of the cows of each group; the tests being collected and classified as to whether minus or plus and as to whether 0.3 per cent or less or over 0.7 per cent. In making this table

the tests for the days on which the milk was left in the udder are not considered. The two-day periods for the normal period are taken consecutively starting with the first and second days, then the third and fourth days, etc. In the experimental periods the two-day periods are all figured from the days on which the milk was left in the udder, going in both directions.

Two-day variations

PERIOD	Total	VARIATION											
		0.3 per cent or less						Over 0.7 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	112	31	27.7	47	42.0	78	69.7	5	4.5	5	4.5	10	8.94
I	72	25	34.7	27	37.5	52	72.2	3	4.2	2	2.8	5	6.95
II	60	25	41.7	19	31.7	44	73.3	1	1.7	1	1.7	2	3.34
III	59	22	37.3	22	37.3	44	74.6	0	0.0	1	1.7	1	1.70
IV	20	3	15.0	7	35.0	10	50.0	0	0.0	0	0.0	0	0.00

A study of this table shows an increase in the variations of 0.3 per cent or less and a decrease in the variations of more than 0.7 per cent in the periods during which the normal milking was interfered with.

A study of the graphs illustrating the variations of the average two-day test (given in figures 1, 2 and 3) does not reveal that the normal tendency of the test of a cow's milk to vary up and down is interfered with. If a rise in the test is due to come it comes, while if the tendency is toward a decline, a decline occurs.

The Official Test is based upon the supposition that the two-day test as taken represents very closely the average test of that cow for the month. To what extent does leaving the milk in the udder cause the test of the milk for the two-day period to vary from the average test for the month more than it normally would. The following table shows the total variations of the average two-days tests of the cows of each group from the average monthly test of each cow; grouping these tests as to whether 0.3 per cent or less or more than 0.5 per cent.

Variation of average two-day tests from average test for the month

PERIOD	Total	VARIATION											
		0.3 per cent or less						Over 0.5 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Numbar	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	120	49	40.8	45	37.5	94	78.3	4	3.3	7	5.8	11	9.16
I	84	31	36.9	35	41.7	66	78.6	3	3.6	6	7.1	9	10.7
II III	130	56	43.1	50	38.5	106	81.6	2	1.5	3	2.3	5	3.85
IV	28	10	35.7	10	35.7	20	71.4	1	3.6	1	3.6	2	7.14

In this table periods II and III are combined as they deal with the same cows. It will be noticed that a somewhat larger percentage of the variations in period I are over 0.5 per cent than is the case in the normal period. Three of these increased variations came before any milk was left in the udder at all and one increase came the fourth two-day period after leaving milk in the udder. There were three minus variations of over 0.5 per cent; one coming before any interference with the normal milking and one just before leaving the milk in the udder and one just after. We can thus account for a large number of the larger variations as taking place at a time when the milk left in the udder had no influence. On the whole the figures show decidedly less variation of the average two-day tests from the average monthly test in those periods in which the milking was not normal. Twenty-two of the average tests for the two-day periods after leaving the milk in the udder were greater than the average test for the month while twenty-six were less.

Throughout the whole trial the milk was left in the udder a total of 48 times. Twenty-eight times the average two-day test for the first two days after leaving the milk in the udder was greater than the test for the two-days before leaving the milk in the udder, and twenty times it was less. Seventeen times it was greater by less than 0.2 per cent and 12 times less by less than this amount.

Let us make further comparison of the average test for the two-day periods before and after leaving the milk in the udder. Adding the yield of milk and of fat for the first two days before leaving the milk in the udder of the first trial in periods I, II, and III and dividing to obtain the average test we have 4.779. Doing the same for the first two days after leaving the milk in the udder of the same trials we have 4.942. There is a difference of 0.163, greater after. Including the second and third trials of period II, which trials are separated from the previous trial by a period of eight days, we get a test of 4.950 before and one of 5.025 after, a difference of 0.075.

If we compare the third and fourth days before leaving the milk in the udder of the first trial of each period with the first and second days after we have 4.931 and 4.942, a difference of 0.011, greater after. Including the second and third trials of period II and using the third and fourth days before we get 5.047 before and 5.025 after, less after by 0.022.

The only change in the production of the cow that occurred consistently in this trial after leaving the milk in the udder was an increase in the flow of milk at the next milking. The cow always came back to normal on the second milking after. Nature has taken care of man's interference with her work and from then on the amount of milk secreted seems to be normal. On the average the increase in the amount of milk after will equal that amount left in the udder. The test of the milk for butter-fat was sometimes more, sometimes less, and again evidenced no change at all. In our work no great advantage in test can be said to have resulted because of leaving the milk in the udder, and many times the test was lower. If milk was left in the udder we had ample proof in the amount obtained at the next milking compared with the other milkings of the two days following.

SUMMARY OF MINUTES OF MEETING OF SOUTHERN DIVISION, AMERICAN DAIRY SCIENCE ASSOCIATION, BIRMINGHAM, ALABAMA, JANUARY 10, 1924

J. A. GAMBLE

Maryland Agricultural Experiment Station, College Park, Maryland

Received for publication April, 1924

FRIDAY AFTERNOON, 2 P.M.

(a) Meeting opened by Professor Wylie, presiding.
President gave opening address.

(b) Moved that reading of minutes be dispensed with for the time. Also committee reports. *Carried.*

(c) Professor Borland stated that dairy industry exceeds the value of beef cattle plus twice hogs, plus twice sheep, in whole country.

Gave an outline of general instruction in more important institutions. He stated that the trend is to give all instruction in Dairy Husbandry Department.

One fourth of dairy cattle in U. S. is on Southern farms, also one sixth of the dairy products and one fifth of all milk.

He called attention to low farm butter prices in Southern States.

He also called attention to the low production of dairy cows in South. Called attention to need for college trained men in Dairy Manufacturing and surveyed the number of men taking dairying in colleges.

How Best to Fit or Train Men:

(1) Biggest factor is the teacher. Outlined the qualifications needed in such a man.

(2) Thorough training in fundamental subjects needed by students.

(3) Courses given will depend on number of students—facilities—instructors.

(4) Preservation of subject matter.

Best to use good texts and mimeographed notes.

Visual instruction should play large part—photographs, slides, charts, etc.

Proper dairy laboratories necessary.

(5) Summer practice should be required before graduation.
(6) Encourage investigation.
(7) Should keep in touch with graduates. Letter or start a round-robin.

(d) C. A. Hutton on Cow Test Association Work in South. (Paper submitted.)

Discussion on how to get testers—Suggested that more money be paid testers.

(e) Prof. J. A. McLean: Address on Feeding Dairy Cattle in South.

(1) Dairy barn and farm is a manufacturing plant and same principles apply as in any other plant.
(2) Four major factors—man, good cattle, good feeding, marketing.
The man should have greatest efficiency.
(3) Feeding is considered by speaker as most important factor of all.
(4) Ideal Feeding:
(1) Produce all feeds at home, particularly legumes and bulky feeds.
(2) Grow proper crops.
(5) Briefly outlined necessity of marketing by-products of milling processes.

(f) L. S. Edwards.

EVENING SESSION, 7:30 P.M.

(g) Paper on Improving Dairy Conditions in South, J. H. McClain.

(h) Report on Dairy Manufacturers Committee by L. S. Edwards. *Adopted.*

Mr. Thompson, Dairy Division, U. S. D. A. read an extract from a speech made by W. F. Jensen at American Association of Creamery Butter Manufacturers, at Chicago, December 4, 1923.

(i) Report of Committee on Nomination read and adopted.

(j) Motion made:

That the by-laws be amended to read that the election by marked ballot of the officers of the Section, be made within one month following the Annual Meeting. *Carried.*

Ayes: LaMaster, Thompson, Edwards, Borland, Thomas, Bennett, Arey, Baer, Hutton, Moore, Brintnall, McLain, Wylie, Holdaway.

(k) Paper read by Earl Brintnall. The Official Testing of Dairy Cattle. (Given elsewhere in this issue.)
(l) Report of Official Testing Committee, Professor Moore, read and adopted.
(m) Report of Extension Committee, C. A. Hutton. *Adopted.*
(n) Report Committee on Instruction in Dairying, J. A. LaMaster. *Adopted.*
(c) Dairy Production Committee, Stanley Combs reported.
(p) Judging Contest Committee.

Names of those present

John Aigdon, C. of Ga....................................Columbus, Georgia
J. S. Moore, A. & M. College....................................Mississippi
Eugene Baker, A. & W. P. RR....................................Atlanta, Georgia
H. L. Alsabrock, A. B. & A. Ry....................................LaGrange, Georgia
S. W. Heath, Dist. Agent....................................Gainesville, Florida
H. G. Clayton, Dist. Agent....................................Gainesville, Florida
J. A. Evans, Asst. Chief Officer....................................Extension Work
Hamelin L. Brown, Dairy Ext. Agt....................................Gainesville, Fla.
J. P. LaMaster,....................................Clemson College, S. C.
L. S. Edwards....................................Lebanon, Tenn.
C. A. Hutton....................................Knoxville, Tenn.
J. A. McLean....................................Chicago, Ill.
J. A. Arey, Dairy Ext....................................Raleigh, N. C.
D. H. Upshaw, Ga. RR....................................Atlanta, Ga.
A. C. Baer, Oklahoma A. & M. College....................................Stillwater, Okla.
W. H. Eaton....................................Auburn, Ala.
Earl Brintnall, A. & M. College....................................Mississippi
G. W. Humphrey, Sou. Ry....................................Atlanta, Ga.
H. S. McLeredon, F. E. C. Ry....................................St. Augustine, Fla.
G. E. McWhorter, Agri. Agt. C. of Ga. Ry. Co....................................Milledgeville, Ga.
S. C. Thompson, Dairy Division....................................Washington, D. C.
J. L. Thomas....................................College Station, Texas
Frederick W. Bennett, Ga. State College Agri....................................Athens, Ga.
J. H. McClain, Dairy Division, U. S. D. A....................................Washington, D. C.
Mrs. J. K. McDowell, Home Economics Dept.........Soft Wheat Millers, Assoc., Nashville, Tenn.
J. K. McDowell.............Feed Dept. Ballard & Ballard Co., Louisville, Ky.
Dr. Tait Butler....................................Progressive Farmer
C. E. Wylie....................................Knoxville, Tenn.
C. W. Holdaway....................................Blacksburg, Va.
A. A. Borland....................................State College, Pa.
Stanley Combs....................................N. C.

RESOLUTIONS OF THE DAIRY SECTION TO ASSOCIATIONS OF AGRICULTURE WORKERS

Resolved: That this Association go on record as favoring the redoubling of the efforts of all interests toward completing the eradication of ticks which at present are retarding and preventing the development of dairying in large areas of the Cotton Belt.

Further resolved: That every effort be put forth to encourage and advance the eradication of tuberculosis and the control of contagious abortion in dairy herds.

Further resolved: That the efforts for developing the creameries in the South be especially directed along quality lines.

Further resolved: That it is more important to increase the production of the present cow population in the South by better feeding than to increase the number of cows or dairy farmers, to this end, i.e. urge the agronomist and county agent forces to put forth special effort to get produced an adequate supply of home grown feeds including pastures.

J. A. GAMBLE,
Secretary and Treasurer.

REVIEW OF FOREIGN DAIRY LITERATURE

H. A. BENDIXEN

University of Idaho, Moscow, Idaho

DRUGE, F. Two Short Researches on Milk. Lait, vol. 12, 1922, p. 101–103.

1. The effect of chloroform and toluol on rennet coagulation: Fresh morning's milk was placed into sterile Erlenmeyer flasks. To one sample was added chloroform at the rate of 14 grains per liter, to the second toluol at the same rate and the third was left without any addition. Each sample then received 1 mgm. of rennet per 100 cc. of milk and was held at a temperature of 34 to 35°. Complete firm coagulation took place in 122, 92 and 87 minutes respectively. Chloroform therefore inhibited rennet coagulation more than toluol.

2. The effect of chloroform and toluol on the spontaneous coagulation of cows' milk: One row of samples remained at 18°, the other at 5°. Chloroform plainly inhibited the action of the lactic acid bacteria; toluol less so. Milk may be kept five days by the addition of chloroform and the application of cold without increasing the acidity.—Matouschek (Vienna).

CATFOLIS, EM. Les présures microbiennes. Compt. Rend. Soc. Biol., Paris, vol. 87, 1922, p. 381–383.

Bacteria coagulate milk with the production of lactic acid following the production of a rennet like enzyme which is also secreted in casein free media. The enzyme production is a normal function of the cell. This bacterial enzyme produces anti-bodies in animals and the bacterial rennet enzymes are different among their kind and also different from the animal rennet enzymes. Differentiation is possible by means of the anti-serum.—Matouschek (Vienna).

RAHN, OTTO. The Importance of Surface Tension Facts in the Dairy Industry. Kolloidzeitschrift, 30, 1922, p. 341–346.

Special experiments to force one to conclude the existence of a film substance in milk not as yet demonstrated in pure form, but which presumably is of protein nature and which may at once explain the formation of milk foam, whipped cream, butter, the skin on heated

milk and the light scorching of milk. The fat in the butter forms no continuous structureless phase. During the initial drying up of the milk the film surrounding the fat globules which consists of the formed film substance is directly visible under the microscope.—Matouschek (Vienna).

WILLE, JOHANNES. Biological and Physiological Observations and Experiments on the Cheese Fly Larva; Piophila casei L. Zool. Jahrb. Abt. f. Allgem. Zool. n. Physiol. d. Tiere., Bd. 39, 1922, S. 301–320, m. 4, Textabb.

In combatting animal pests it is of great scientific interest, to determine which organs a combat material attacks and what changes they experience. Naturally therefore a thorough investigation of the biology and physiology of each pest must precede. With a gassy combat substance the respiratory organs are most important and besides, for example, the diffusion through the skin and absorption by the blood or the body cavity fluid. Here naturally the physical conditions of the gases and their chemical composition are most important for the action upon the animal body as well as the consideration of injury to colors, fabrics, metals, food materials, etc. when using gas. Besides the knowledge of the normal life processes of the pests is necessary to examine into the pathological conditions and irritations by the gases.

If for instance gas is allowed to act upon the cheese fly larva which is so detrimental to cheese, quite definite effects result on the larvae which are manifested by restless crawling around, jumping, etc. The task of the author was therefore to observe the several stimulations on the Piophila casei and to draw conclusions from these as to the biology and physiology of the insect.

The results of his studies were:

1. The crawling of the cheese fly larva is an alternating push and pull movement.

2. The forward movement by jumping is not found during the larva stage I and II, but only in the larva stage III and then most frequently and plainly with larvae which will pupate in a day.

3. The larvae III show negative phototropism.

4. As morphologically important parts of the larva body for the skipping process must be considered the head and posterior parts, which show special differentiations. These special parts are of greatest importance for the details of the skipping process.

5. The skipping process consists of bending the body together,

anchoring both body ends, stretching, and snapping off. The motion in skipping is in the direction of the head. The essentials for the jump are the muscle tensions in the larva body, the longitudinal muscles exerting a pull and the ring-muscles a pressure. The effect of the jump is increased by the initial stoppage of movement. A glueing function by means of special secretions does not exist.

6. As stimulations for the skipping process were experimentally determined mainly the influence of the light, in second place the influence of moisture. Temperature stimulations, air currents, mechanical and chemical stimulations are of no significance for the skipping.

Kickinger, H. The Decomposition of the Citric Acid of Cows' Milk by Some Bacteria. Biochem. Ztschr., Bd. 132, 1922, S. 210.

Comparing the experimental results ascertained by the author the following conclusions seem justified.

1. The citric acid content in pasteurized and boiled cows milk is the same when determined immediately after heating the milk as when the milk is fresh; however, it decreases more or less on prolonged standing. In fractionally sterilized cows' milk as compared with fresh milk the citric acid shows a rather marked decrease during the first days. After the third sterilization, however, the citric acid content remains constant.

2. The cause of the decrease of the citric acid is no doubt found in bacteria and in the experiments under consideration they were representatives of the group of peptonizing bacteria (Bac. subtilis) Bac. mesentericus vulgatus and Proteus vulgaris), while lactic acid formers (3 different species, as well as a species of Joghurt bacilli) had no effect on the citric acid of the cows' milk.

3. The enantimorphic action of some lactic acid formers on one side and the peptonizing bacteria on the other allows with only slight probability of accuracy to draw conclusions as to the kind of bacteria from the amount of the citric acid present.—Heuss (Berlin).

Frog, F., and Schmidt-Nielsen, S. The Fatty Acid Content of Butterfat. Biochem. Ztschr., Bd. 127, 1922, S. 168.

The fatty acids of the examined butterfats were as follows:

	per cent
Butyric acid	3.4
Caproic acid	3.3
Caprylic acid	1.9
Capric acid	3.0

Lauric acid	3.7
Myristic acid	12.9
Palmitic acid	20.8
Stearic acid	6.2
Oleic acid (maximum values)	27.0
Not definitely identified acids (Gadoleic acid?)	
Linoleic acid?, acid $C_{20} H_{30} O_2$? arachic acid	
Behenic acid?	9.8
Distillation remnants	8.0

—Heuss (Berlin).

Bleyer, B., and Seidl, R. Contributions to the Knowledge of Cows' Milk Casein. Biochem. Ztschr., vol. 128, 1922, p. 48–75.

In fresh milk the casein which is almost insoluble in water is combined with CaO as "Söldners Casein-lime Compound" which is very strongly dispersible in water and which forms with it colloidal solutions, salt solutions. To separate the casein from the milk there are two methods available:

Forcing the casein out of its natural state by means of acidulation and by precipitating by means of the rennet enzyme. The laboratory experiments showed in regard to composition only a slight difference between acid- and enzyme casein; under the conditions at hand the former proved much more active than the latter. There is something conflicting. The authors first sought a casein as nearly as possible free from ash by a carefully described method. The N-factor of the acid casein is 100:15.5 equal 6.45, that of the paracasein 100:15.64 equal 6.39. Each gram of these two substances required 8.74 cc. of base for neutralization with phenolphthalein, which gives an acid equivalent of 11.45. Both caseins react with the alkaline earths according to the law of Henry, but they are not true compounds. The acid casein and paracaseins respectively result from adsorption, and the greatest adsorptive ability occurs with HCl. The paracasein can take up more acid than the casein.—Matouschek (Vienna).

DAIRY NOTES

Dairy Division, B. A. I. United States Department of Agriculture

Jackson, H. C., a graduate of Cornell University, and with a Ph.D. degree from that institution, has been appointed for research work in the manufacture of dairy products and by-products. Mr. Jackson has served as assistant professor in dairy manufacture at Cornell University, and has had practical work in plants manufacturing powdered milk, condensed milk, butter, cheese and market milk.

Frazier, William C., a graduate of the University of Wisconsin, and with a Ph.D. degree from that institution, has been appointed for research work in the bacteriology of milk. Mr. Frazier has served four and one-half years as instructor in the University of Wisconsin. For two years he was with the American Expeditionary Force, a part of the time working on the diagnosis of various diseases at the Central Medical Laboratory, Dijon, France.

THE REVIVAL OF DAIRY RESEARCH IN GERMANY

Agricultural research, particularly dairy research, revived vigorously in Germany during the past year. The first attempt after the war to reëstablish this important work failed. This condition was attributed to the breakdown of the German finances. Forschungen auf dem Gebiet der Milchwirtschaft, a scientific dairy journal was published for a period of eighteen months during this depressing time and discontinued.

At present the German government maintains a pure dairy research station at Kiel. This institution has six departments, and is well equipped and has just been reorganized. Eastern Germany offers opportunity for dairy research at the Institution of Research at Königsberg. It is at this institution that Dr. Grimmer edits a new and permanent dairy research journal, Milchwirtschaftliche Forschungen.

Government dairy research activities are also carried on at Halle by Professor Gutzeit; and at Weihensphan (near Münich) by Professor Fehr.

The above mentioned institutions have connections with the universities. Besides these there are other stations receiving private and

government support in Germany. These, however, do not have a direct connection with the universities.

Another step in the promotion of agricultural research has been the establishment of a research branch for agriculture at the University of Breslau. This project has included facilities for housing the new work which cannot be surpassed. The main building is as large and well constructed as buildings used for a similar purpose at universities in this country. The main new building at Breslau includes general offices, laboratories for the study of biochemistry, veterinary science, and agricultural sciences. Greenhouses, barns and other buildings have been provided for the work which will be conducted at this institution.

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Below is published a list of the paid up membership of the American Dairy Science Association for 1924. Any reader of the JOURNAL, elegible to membership, whose name does not appear in this list should communicate at the earliest convenience with Secretary J. B. Fitch, Manhattan, Kansas. Prompt remittance of dues will insure the receipt of every number of the JOURNAL. It will also avoid unnecessary changes in committee appointments. Send your remittance today.—*Editor.*

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YEAST AS A SUPPLEMENTARY FEED FOR CALVES

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The importance of vitamins to human and animal nutrition is generally accepted. It is not so clear, however, as to the extent to which generalization based upon observation with one species can be applied to another. The rat and the prairie dog for example appear able to thrive without the antiscorbutic factor, or have the power of producing the substance synthetically within the body. The guinea pig on the other hand is very sensitive to a lack of this vitamin.

For this reason the question arises as to the extent to which results with laboratory animals can be applied to the larger domestic animals. So far investigations concerning the vitamin requirements of the larger domestic animals are exceedingly limited and it would appear a considerable proportion of those which have been reported may in addition have involved questions of mineral metabolism and a possible deficiency of some mineral elements.

Swine among domestic animals have received the greater amount of attention. The results with this species are somewhat conflicting but do not on the whole indicate any serious dangers from a deficiency of vitamins under practical conditions. Morrison and associates (1) have shown that yellow corn is superior to white corn for swine when no other source of vitamin A is present. On the other hand Orr and Chrichton (2) in a more recent report indicate no effects were apparent from feeding pigs for a period of 110 days on rations essentially free from either vitamin A or vitamin B.

The experimental data available regarding the relation of vitamins to the nutrition of cattle center around the question of the relation of the antirachitic factor to the assimilation of calcium and the disease known as lamziekte prevalent in South Africa.

Hart, Steenbock and associates (3) have made an important contribtuion indicating a relation between a fat soluble vitamin and the assimilation of calcium by the lactating cow.

The work of Theiler and associates (4) indicates that the disease known as lamziekte is the result of a toxin somewhat similar to the botulinus toxin and which is formed by organisms growing in the bones of dead cattle. As a result of a serious mineral deficiency of the cattle due in turn to a soil low in minerals, the animals develop "pica" or abnormal appetite and eat bones and other parts of the carcasses of dead animals containing this toxin.

This disease is of interest here on account of the fact that while it was under investigation prolonged tests of rations low in vitamin were made with cattle. Cattle were kept for a year on a ration consisting chiefly of white rice with no evidence of beri-beri symptoms. Extracts rich in vitamin B were also fed without any effect. One cow fed white rice and autoclaved straw gave birth to a blind calf. Theiler suggested that the ability of the animal to survive without vitamin B in the ration might be due to the formation of the vitamin in the digestive tract as the result of a bacterial symbiosis.

Significant data pointing towards this possibility have been published recently. Damon (5) found that the addition to the ration of desiccated preparations of certain bacteria including B. timothy (the timothy grass bacillus of Moeller) served to supply the vitamin needed for the growth of rats. Scheunert and Schierlich (6) report that B. vulgatus (Flügge) Migula, an organism which grows rapidly in the intestines of herbivora, is able to synthesize vitamin B in a vitamin free medium Pigeons were used in making these tests.

Another point to be considered is that the ration as received by cattle under ordinary conditions is mixed in character, including usually ground seeds and especially a large amount of roughage.

Considering the nature of the ration the danger of a deficiency of vitamins in the nutrition of the bovine would appear remote. However, the present tendency is towards the feeding of more by-products, especially of the milling industry, and this increases the possibility of such a shortage occurring.

Pronounced effects have been reported from feeding vitamin B in the form of yeast to laboratory animals. Hawk and others have also reported improved health in humans following the administration of yeast. These results suggest the possibility that yeast might have some value as a supplementary feed for calves.

The large quantities of spent yeast, a brewery by-product available from breweries, presents an opportunity for increased profits by finding a suitable means of disposal and has lead to a considerable amount of experimental work. The use of this by-product as a feed for livestock appeared to be the best solution and for a number of years the use of spent yeast as a feed for farm animals, especially cattle, has been common. Among those contributing to the development of methods of preserving this by-product were Schmidt (7), Makin (8), Hayduck (9), Fallada (10), Herzfield (11), and Miller (12).

The variety of methods followed in the preparation of this material for feeding purposes naturally results in a wide range in the composition of the product as used for feeding purposes. Czadek (13) gives the following analysis and digestion coefficients for a yeast feed used in his experiments.

	PER CENT	DIGESTION COEFFICIENT
Moisture	10.90	
Protein	55.60	90.8
Ether extract	0.53	62.5
Nitrogen free extract	18.90	76.4
Fiber	5.85	
Ash	8.31	

It should be pointed out that spent yeast as used for feeding purposes in Europe has been generally considered as a source of protein and energy as would be the case with other more common

feeds and not on account of any special dietetic or nutritional value. The experiments reported show in general that dried spent yeast is a suitable feed for cattle and is the source of a highly digestible protein supplement.

RELATION OF VITAMIN B TO GROWTH

Vitamin B is often referred to as a "growth promoting vitamin or accessory" and the necessity of this vitamin for the growth of experimental animals is now generally accepted.

Sherman and Smith (14) after reviewing the whole field of investigations with vitamin B state that the absence of B from the diet causes cessation of growth; that a partial but not complete deficiency in this vitamin leads to impaired growth and a general undermining of health and vigor and this lowered vitality may have a far reaching effect in its influence on reproduction and the successful rearing of young.

Experimental

The fundamental principles of nutrition can unquestionably be studied to the best advantage by the use of small laboratory animals. There is, however, an uncertainty as to what extent these results may be applied to the larger species of domestic animals. With the vast economic importance of the larger domestic animals it becomes a matter of great concern to determine to what extent the findings from the small animals apply and especially if the practice of feeding as now followed results in serious economic losses.

It would appear to be the function of the agricultural experiment stations to make these applications suggested. With this object in mind investigations have been undertaken for the purpose of studying one phase of this subject, that of the relation of vitamins to the growth of calves. In planning these studies the logical procedure appeared to be: (a) to determine if the growing calf requires the same vitamins as has been found necessary for the growth of laboratory animals; (b) an estimation of the quantitative requirements in terms of some feedstuff serving as an espe-

cially potent source of the vitamin in question; (c) to determine if there is any danger of rations as ordinarily used in practice being deficient in the vitamin in question; (d) if there is any advantage in giving a surplus of the vitamin.

The first to receive our attention was the B vitamin. This vitamin was selected for study on account of the fact that yeast, a potent source of vitamin B, is available in large quantities as a by-product from breweries and yeast factories. Moreover the work of Hawk and associates (15) has aroused a wide spread interest in the use of yeast in the human dietary and attempts are being made to introduce yeast products as supplementary animal feeds. Furthermore assistance in financing the investigation was available from a commercial organization interested in yeast products. The logical procedure would be to develop the study in the order suggested in a previous paragraph. The opportunity to secure finanical aid, however, made it advisable to first determine if any advantages could be found from the addition of yeast as a supplementary feed to rations such as are commonly used in practice. If beneficial results are possible from yeast feeding it seems safe to assume that the growing calf and the cow in milk would profit most. For this reason our experiments included calves from birth to six months of age and lactating cows. The results from the latter will be reported in a later paper.

While milk is not generally looked upon as an especially good source of vitamin B the fact is that on the dry matter basis it is perhaps in the same class as seeds and can be looked upon as a good source of this vitamin. It has been demonstrated that the amount of vitamin B present in milk is dependent upon the amount supplied with the ration and consequently varies from time to time. Results from adding yeast to the ration of calves were expected to show a stimulation of the rate of growth, or in a more healthy condition of the animals.

Plan of experiment

The first series included nine calves divided into three groups and receiving rations as follows:

Group 1. Whole milk, grain and hay to 180 days. Limitations placed on grain and hay. Dried bakers' yeast fed intermittently during the period.

Group 6. Skimmilk to 60 days with addition of grain and hay. Dried bakers' yeast was added for prolonged periods up to 180 days.

Group 3. Skimmilk, grain and hay to 180 days. Dried brewers' yeast fed intermittently.

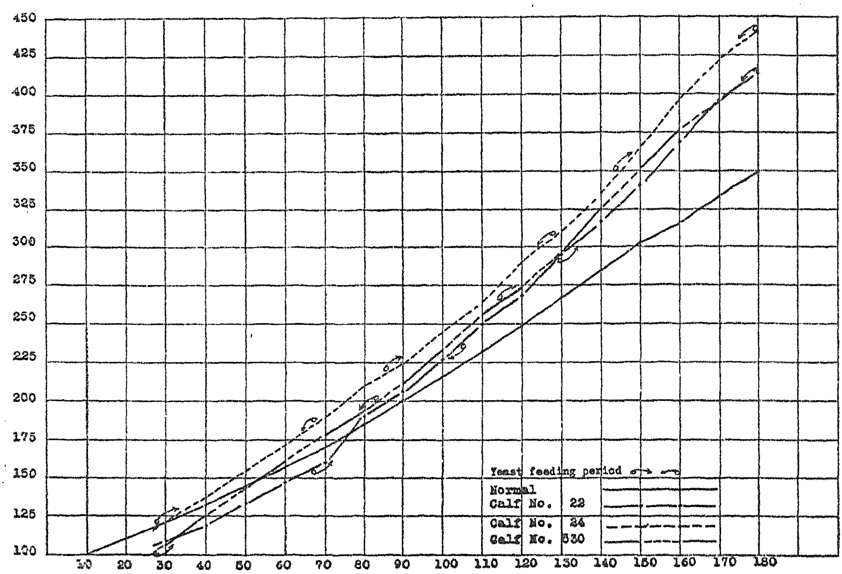

FIG. 1. THE RESULT OF ADDING YEAST TO A RATION INCLUDING WHOLE MILK

These three calves made a gain in weight above the normal as a result of the whole milk received. The yeast feeding, which is indicated by the arrows, did not make any noticeable increase in the rate of growth.

In arranging these groups it was planned that group 1 would represent conditions as found in purebred herds where the best ration is fed regardless of economy. Group 2 represented the plan of raising calves according to the "minimum milk" plan which has been under investigation by this experiment station for several years, and group 3 represented typical rations used when the raising of calves on skimmilk is practiced.

In this series the yeast used was of the kind sold as bakers' yeast. It was received in large lots and dried at a low temperature as a means of preservation. The air dried product contained about 50 per cent filler in the form of starch. It was fed in the dry form in quantities equal to 120 grams of fresh bakers' yeast daily to each experimental calf, a quantity three or four times the amount a human being might consume. The periods of yeast feeding are shown by arrows on figures 1 and 2.

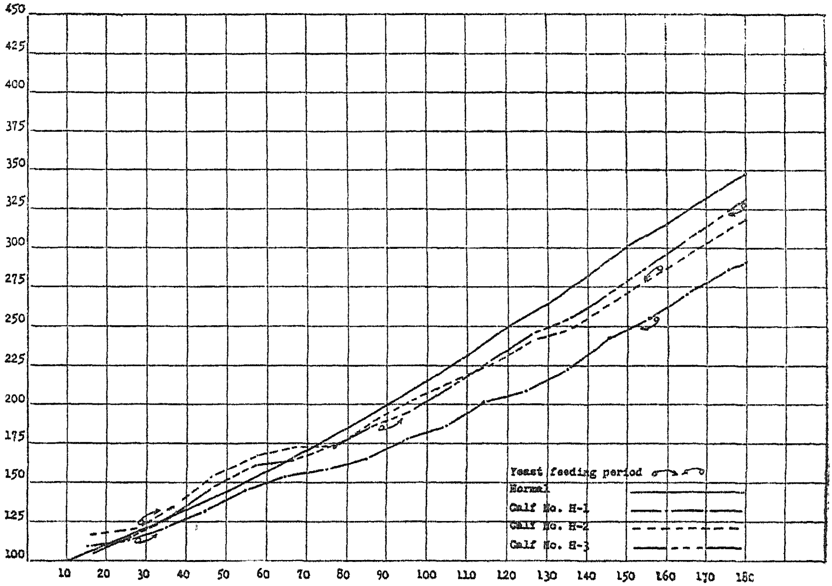

FIG. 2. THE RESULT OF ADDING YEAST TO A RATION FED CALVES WEANED AT SIXTY DAYS

These three calves received a ration including skimmilk to the age of 60 days, with grain and alfalfa after that age. The yeast was added during the intervals indicated by the arrows. The yeast feeding had no noticeable effect upon the rate of growth.

No appreciable effect could be seen on the appetite of the animals or the rate of growth. The calves, with the exception of one that contracted pneumonia and died, remained in excellent health and were perhaps more than usually vigorous.

Experiments with dried commerical yeast

The second series of experiments was conducted for the purpose of testing the value of commercial dry yeast as a supplement to the rations of growing calves. The yeast used is sold in large quantities in the dry form, the yeast cells being mixed with ground grains, apparently mostly cornmeal. The following is the chemical analysis of the yeast product used.

	per cent
Ash	10.60
Crude protein	1.75
Ether extract	11.65
Crude fiber	1.04
Nitrogen free extract	71.06
Total	100.00

The analysis indicated that the yeast product contained about three per cent of yeast cells. The drying process appeared to be satisfactory since microscopic counts of the dried yeast showed up to one hundred twenty billion live yeast cells per gram of material. The results of the tests of the yeast as a source of vitamin B for laboratory animals is given in a later paragraph.

PLAN OF EXPERIMENT WITH GROWING CALVES

Our aim was not to determine the physiological effects of yeast feeding but rather to study the question from the viewpoint of the feeder of dairy cattle. The results were measured from the practical feeder's standpoint in terms of growth as indicated by skeletal development, gain in weight, health as indicated by general appearances, and freedom from disease. The rations ordinarily fed calves in the better herds include such feed stuffs as legume hays, milk, corn, and cereal grains, substances which are good sources of vitamin B. If the requirements of cattle for this factor approximate those of laboratory animals in relaton to food intake there should be little danger of a deficiency occurring. If these assumptions are correct the experiments reported should indicate not the necessity of vitamin B in the ration but rather whether the addition of an extra amount of this vitamin in a potent source like yeast will have any favorable effect.

A total of 38 calves were used of which 18 served as check and 20 received the yeast supplement. Three groups were used with the general plan of feeding as follows:

Group 1. Yeast was added during the year to the rations of half the calves born in the University purebred dairy herd. These calves were otherwise raised in the ordinary manner.

Group 2. A second group composed of grade calves was fed skimmilk, alfalfa hay and grain mixture of corn, wheat, bran, and oilmeal. The rations received by part of these calves were supplemented with yeast.

Group 3. The rations of a third group consisted of skimmilk, prairie hay and a grain mixture of corn and oats. The rations of half of this group were likewise supplemented with yeast.

Group 1. The purpose of this experiment was to determine if there is any advantage in adding yeast to the ration of calves receiving the treatment usual in well managed herds where the calf has considerable value and for this reason received especially good treatment. The results secured would be most applicable to purebred herds.

The purebred calves born in the University herd during the course of a year were used. They were placed in the experiment at birth and arranged in pairs according to breed and sex. One animal of each pair received 100 grams of the dried yeast daily in addition to the regular ration. Eleven pairs were so arranged and compared. Records of the weights and heights at withers were taken three days each month. No record was kept of feed consumed, otherwise than of the amount of yeast. The herdsman was instructed to follow his usual routine except to add the yeast supplement to those designated. The results are shown in figure 3 in which the averages are given for the groups compared to the normal. It will be noted that those receiving the yeast made a slightly better growth during the latter half of the period. The check group finished 104.5 per cent normal in weight and 101.9 per cent in height. The yeast group at the close were 107.8 per cent normal in weight and 102.8 per cent in height. This difference is too small to be of any special significance.

Group 2. This group consisted of eight vigorous grade Holstein heifer calves purchased from a dealer and placed on experiment at about 20 days of age. Of these five were experimental and three controls. This group was to represent typical methods of feeding followed when calves are raised with skimmilk. The skimmilk was fed at the rate of 16 pounds daily with an allowance of

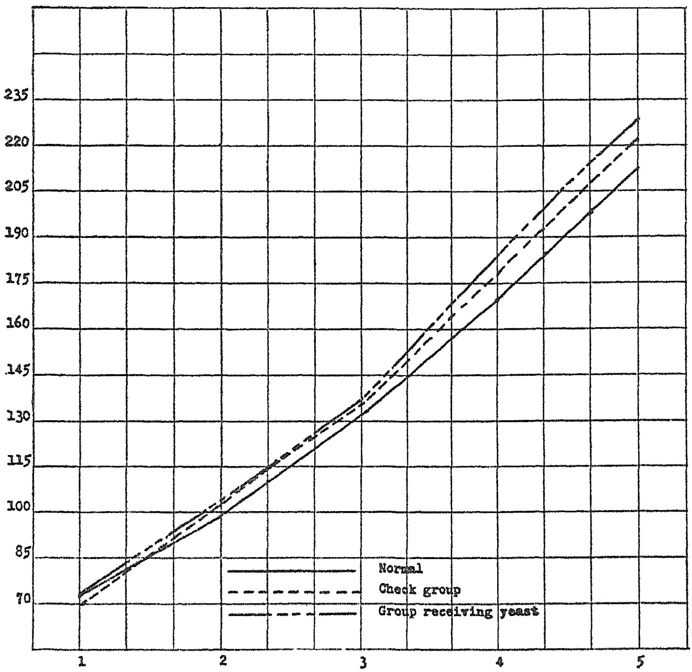

FIG. 3. THE RESULTS OF ADDING YEAST TO THE RATIONS OF CALVES FED OTHERWISE ACCORDING TO THE USUAL PRACTICE IN THE UNIVERSITY HERD

The line marked "check group" shows the average growth as shown by weight of eleven calves receiving the usual calf ration. The line marked "group receiving yeast" shows the average of eleven calves receiving the same ration with a supplement of 100 grams of dried yeast daily.

from 2.5 to 3 pounds of grain, and as much hay as would be consumed. Those receiving the yeast supplement were started at 50 grams daily which was gradually increased to as high as 300 grams. On account of the negative results it is not considered

necessary to give the data in detail. The results are shown graphically in figures 4 and 5 in which the growth of the check and experimental animals is compared to the normal. The results in percentage of the normal are given below.

	CHECK GROUP THREE ANIMALS	RECEIVING YEAST IN ADDITION, FOUR ANIMALS
Per cent of normal weight at beginning	104.6	107.6
Per cent normal weight at 180 days	110.6	103.2
Per cent normal height at beginning	100.4	102.3
Per cent normal height at 180 days	99.8	100.4

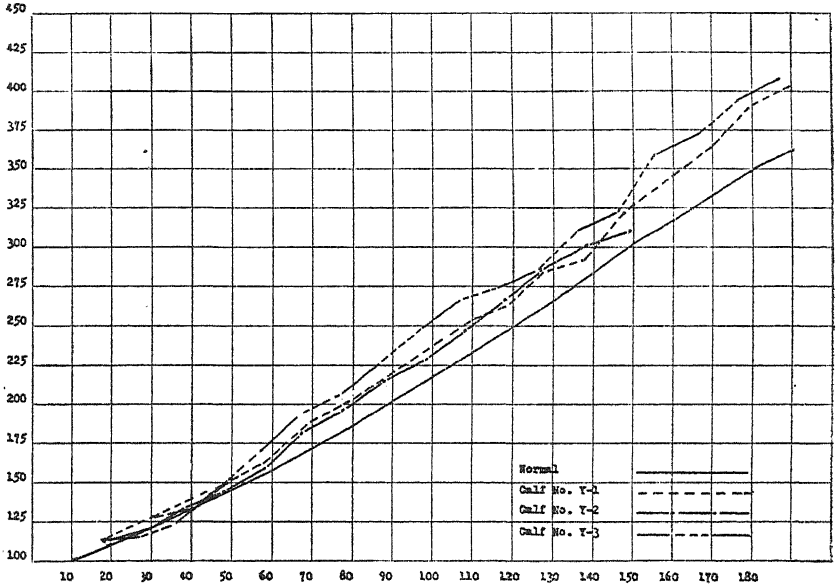

FIG. 4. CHECK GROUPS RECEIVING NORMAL SKIMMILK RATION

These three animals served as checks for those receiving the yeast supplement. The growth of the experimental animals receiving the same ration with a yeast supplement is shown in figure 5. It will be noted that the check animals gained in weight somewhat more rapidly than the normal.

Evidently the yeast did not stimulate the growth of these calves. In fact the check animals made the best growth. The results are considered negative.

Group 3. It was realized that the ration used in group 2 was a good one and one known to give normal results. Under conditions as existing on farms, a considerable proportion of the calves raised do not receive a ration as favorable from the standpoint of composition. For this reason it was decided that the third group should have a ration that would represent a rather inferior ration, but one that would be typical of feeding as often practiced. This ration was prairie hay, corn, and oats. This ration is inferior

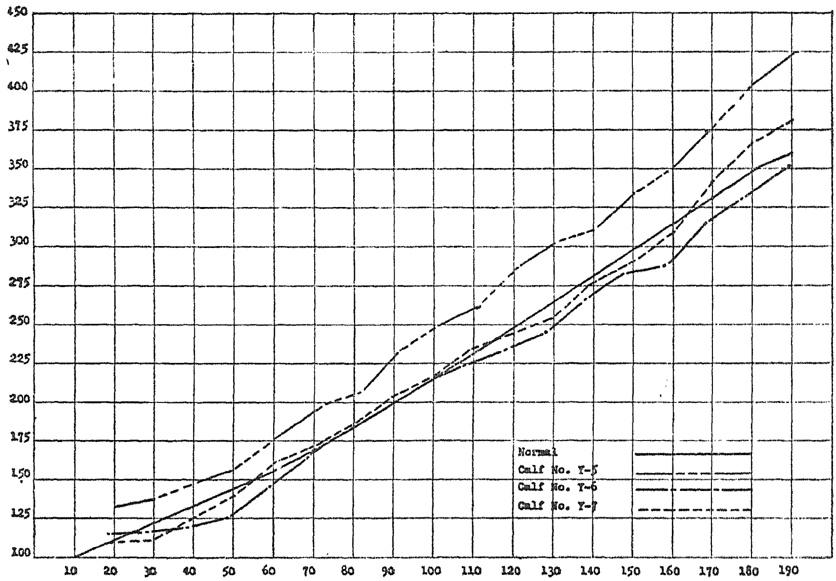

FIG. 5. CALVES RECEIVING 10 PER CENT DRIED YEAST AS A SUPPLEMENT TO A SKIMMILK RATION

The growth of these five animals is to be compared to that of the checks shown in figure 4. No advantage can be observed from the use of yeast. The gains made by the check group without the yeast were somewhat better.

to that used in group 2, in the amount and quality of proteins, in the calcium and phosphorous, and probably in vitamins. However, similar rations are widely used where leguminous hays are not grown and where such feeds as bran and linseed meal are considered too expensive.

The group consisted of eight vigorous Holstein heifer calves. Four were used as checks and four received the yeast supplement.

The skimmilk was fed at the rate of 16 pounds daily with from 1 to 3 pounds of mixed corn and oats and prairie hay at will, the amount consumed increasing from nothing up to about 4 pounds daily at six months of age. The four experimental animals received dry yeast in quantities to supply 10 per cent of the dry matter of the ration. The results are shown graphically in

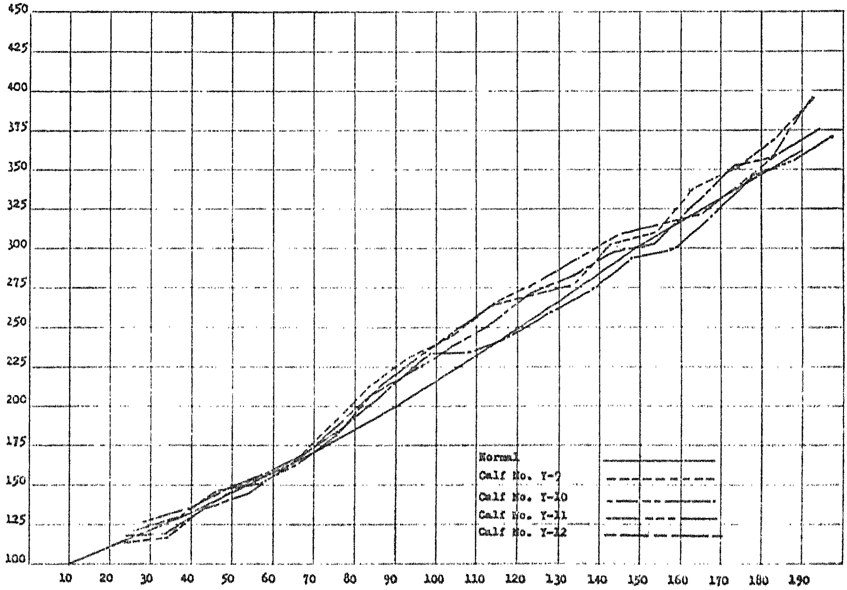

FIG. 6. THE GROWTH AS SHOWN BY WEIGHT COMPARED TO THE NORMAL OF FOUR CHECK CALVES IN A RATION OF SKIMMILK, PRAIRIE HAY, CORN AND OATS

The results from the experimental animals for which these are checks are shown in figure 7.

figures 6 and 7. The following figures show the comparative growth represented in percentages of the normal.

	CHECK GROUP, FOUR ANIMALS	RECEIVING YEAST IN ADDITION, FOUR ANIMALS
Per cent normal weight at beginning................	103.3	105.5
Per cent normal weight at 6 months of age...........	104.3	108.4
Per cent normal height at beginning..................	98.7	100.0
Per cent normal height at 6 months of age............	100.4	102.5

With this group those receiving the yeast supplement made a slightly better gain in both weight and height. However, the difference is not significant and not greater than the advantage shown in favor of the check animals in group 2.

LABORATORY TESTS OF THE YEAST FED

Whatever value yeast may have as a supplementary feed for growing calves is presumably due to the vitamin B. content.

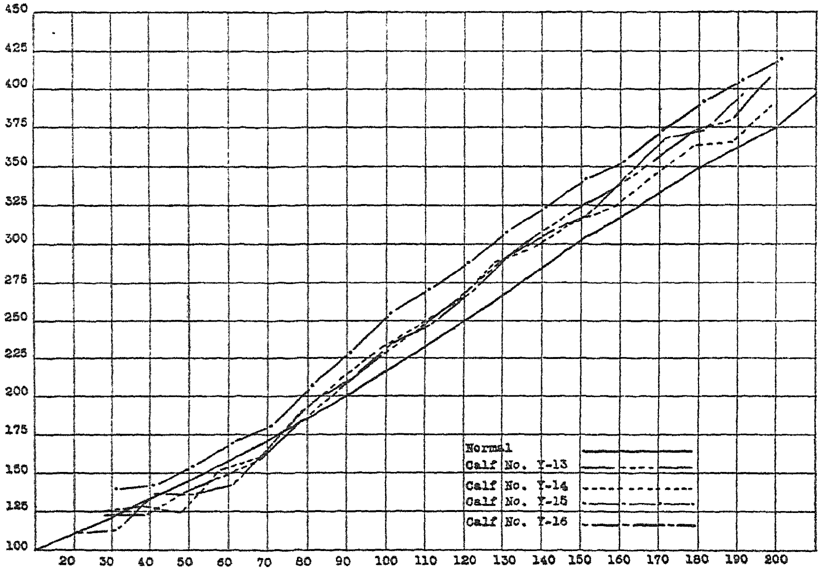

FIG. 7. THE GROWTH AS SHOWN BY WEIGHT COMPARED TO THE NORMAL OF FOUR CALVES RECEIVING THE SAME RATION AS THOSE SHOWN IN FIGURE 6, BUT WITH THE ADDITION OF DRIED YEAST EQUAL TO 10 PER CENT OF THE DRY MATTER IN THE RATION

The yeast did not appear to have any effect upon the growth of the calves

For this reason it appeared advisable to test the yeast used in the second series of experiments as a carrier of this vitamin by small animal experiments. The white rat was used for this purpose.

The specific objects of the small animal experiments were as follows:

1. To determine the level at which it was necessary to feed the commercial yeast preparation in the ration of rats as a sole source of vitamin B, in order to obtain normal growth.

2. To determine the influence, on the growth of rats, of additions of the yeast preparation to rations which were typicalof a good and a poor calf ration.

The methods followed were typcial of those usually employed in work of this kind. The animals were kept in individual cages with a supply of the experimental ration before them at all times. Each cage was equipped with a false bottom of one-fourth inch with wire screen to obviate as far as possible the consumption of excreta. Weekly records of the weights of the animals and of the amount of the feed consumed. The basal ration which was that used by McCollum, except for an increase in butterfat, was composed of the following:

	percentage
Casein	18.0
Butterfat	9.0
Salt mixture	3.7
Agar	2.0
Dextrin	67.3

The mineral mixture given was made up according to formula 185 as given by McCollum.

The various feeds used for the growing calves, including alfalfa, prairie hay, corn, oats, were finely ground in order to make it possible to mix them with the ration successfully.

The potency of the yeast was first determined by feeding six groups of rats the dried commercial yeast at levels ranging from 10 to 40 per cent of the total ration. For some the yeast was substituted in the ration for an equal amount of dextrin, in other experiments the yeast preparation was fed separately. The results showed that a fairly satisfactory growth and reproduction resulted when commercial yeast was mixed with or fed separately in the ration to the extent of from 15 to 20 per cent. Typical results showing the growth curves of rats used are presented in figure 8.

The addition of the commercial dried yeast preparation to the ration of rats as a source of vitamin B resulted in good growth for more than a year and also enabled the rats to reproduce and and in some cases to rear their young. The use of higher levels

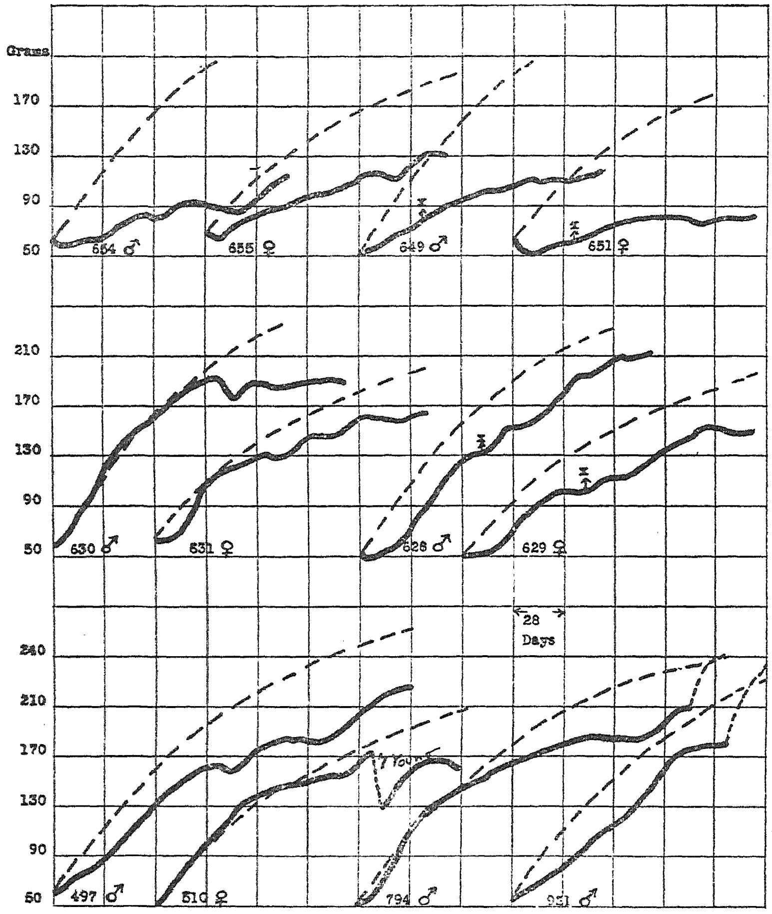

FIG. 8. GROWTH CURVES OF EXPERIMENTAL RATS

Rat 497 received a ration of casein, salts, butterfat, agar and corn starch dextrin containing 10 per cent of dry commercial yeast preparation.

Rat 510 received a like reaction containing 15 per cent of the yeast preparation. The litter of 7 young was successfully reared.

Rat 794 received the same basal ration without the yeast incorporated in it, and was given yeast separately at the rate of 10 per cent of the total food intake. The dotted part of the curve shows the effect of changing the ration to the one fed the breeding colony.

Rat 921, one of the litter of rat 510, was raised on the mother's diet. The dotted part of the growth curve shows the effect of changing the ration to that fed the breeding colony.

of this yeast up to 40 per cent of the ration did not induce any more favorable results. In no case was entirely normal growth obtained and when the ration was changed to one composed of cereal grains, legume seeds, alfalfa, cod liver oil, meat scraps, salts, and milk, the growth of the animals was stimulated and rapidly approached the normal. Several cases of polyneuritis resulted in nursing rats when their mothers received as high as 15 to 20 per cent of the yeast preparation.

Since the yeast preparation used is essentially a mixture of yeast cells and cornmeal it seemed worth while to also compare the yeast preparation with cornmeal as a source of vitamin B. It was found that the 15 per cent of ground yellow corn gave results somewhat inferior to 15 per cent of the dried yeast. Twenty-five per cent of white corn in the ration, as a source of vitamin B allowed rats to make practically normal growth.

Two groups of rats were fed the ration received by the calves in group 2. The ration as prepared for the feeding of the rats was composed of the following ingredients:

	per cent
Alfalfa	24.1
Cornmeal	31.54
Wheat bran	7.88
Oilmeal	7.88
Skimmilk powder	28.60

In addition one group of rats received ten grams of the dry yeast in each 100 grams of the ration. Both groups did well for a time, but finally began to fall behind the normal, due probably to the fact that the rat is not adapted to the utilization of a

Rats 630 and 631 received a diet consisting of alfalfa 24.0 parts, corn 31.5 parts, bran 7.9 parts, oil meal 7.9 parts, skimmilk powder (produced from the Station herd milk) 28.5 parts.

Rats 628 and 629 received the same diet as rats 630 and 631 except that 10 grams of the yeast preparation were added to 100 grams of ration. Beginning at x the yeast was fed separately at the rate of 1 gram daily.

Rats 654 and 655 received a diet consisting of prairie hay 24.1 parts, corn 23.6 parts, oats 23.7 parts, skimmilk powder 28.6 parts.

Rats 649 and 651 received the same diet as 654 and 655 except that 10 grams of the yeast preparation were added to 100 grams of ration. Beginning at x the yeast was fed separately at the rate of 1 gram daily.

ration containing so much bulk. No beneficial result was observed from the yeast. Typical results are shown in figure 8 for rats 630 and 631, which received the ration without yeast and for rats 628 and 269, which received the yeast addition.

The poorer ration fed the calves in group 3 was in like manner tested by feeding two groups of rats a ration essentially the same. The rations as received by the rats was composed of the following:

	per cent
Prairie hay	24.1
Cornmeal	23.65
Oats	23.65
Skimmilk powder	28.6

The ration received by one group of rats contained 10 per cent of the dried yeast. In this case, as with the better calf ration, no advantage was found from the addition of yeast. It was found, however, that the growth of rats on the poor calf ration was decidedly below that of the rats which received the good calf ration. Typical results are shown in figure 8. Rats 654 and 655 received the poorer ration without yeast and rats 649 and 651 the ration with the yeast addition.

CONCLUSIONS

1. The addition of vitamin B in the form of dried yeast to the rations ordinarily fed on dairy farms did not increase the rate of growth of calves from the age of 20 to 180 days.

2. No definite effect was observed on the health of calves as a result of supplementing their ration with dried yeast.

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A STUDY OF THE FACTORS AFFECTING THE GROWTH OF DAIRY HEIFERS[1]

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The many recent discoveries in the field of nutrition have necessitated a change in the methods of attacking nutritional problems. While for many years the chemical analysis of a feed was taken as the true index of its usefulness in a ration, we now know that feeds containing the same amount of protein, carbohydrates, and fat may differ widely in their physiological effect on the animal. Hart, McCollum, Steenbock, and Humphrey (1, 2) in an early study proved that growth and reproduction are seriously affected by limiting the ration of dairy heifers to the product of a single cereal plant. The heifers fed on the corn plant grew well, matured and showed early oestrus, and were physically strong in every respect. Those receiving the wheat ration grew at a fair rate until they reached 1000 pounds in weight, when growth ceased. They showed lack of vigor and evidence of physical weakness; even blindness finally resulted. They showed no oestrus. Similar, though not so marked, were the results obtained from feeding the oat ration. Later experiments bringing forth the accessory food factors and the difference in the quality of proteins have explained the reasons for many of the difficulties encountered in the studies of nutrition.

The fact that these qualities and limitations of feeds cannot be determined by chemical analysis has opened a new field in the study of nutrition. Only by means of actual feeding trials of the many grains, alone and in combination, is it possible to determine their physiological value; which determinations must be made before any constructive advance can be made toward the

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solution of certain problems confronting the dairy farmer of today.

One problem which has been of great interest to investigators for many years is that of reducing the cost of raising calves. Today in sections of the country where whole milk is sold, the problem of raising calves to replace the old and discarded cows, is becoming quite serious. From the beginning of time, milk has been regarded as the food best adapted to the complete nourishing of mammalia, and so far no food has been found which will completely take its place. Partial substitutes have met with a fair degree of success, and supplements to milk have helped to reduce the amount necessary to raise the calf. These are fed however in the form of a slop and require considerable time in preparation; and the best results from their use are obtained only under ideal conditions. Whether or not it will ever be possible to obtain a complete substitute for milk is questionable.

If such a food is obtained it will of necessity be fed in the form of a liquid, due to the nature of the calf's stomach; since a complete substitute for milk would mean a food which will completely take the place of milk at the time when nature intended the calf to be nourished solely on milk. Under experimental conditions this would not detract from the value of the experiment, but under practical conditions the time and exacting details connected with the preparation of such a substitute would probably make it prohibitive. The only alternative in this case would be to determine the earliest age at which a calf may utilize solid food as the sole source of nourishment.

The recent developments in the field of nutrition emphasize the necessity in this case of a thorough study of the various grains with a view of determining which feeds may be utilized by the calf at the earliest age, and to the best advantage. This would mean a thorough study of the factors affecting growth.

PURPOSE OF EXPERIMENT

It was with the idea of studying these factors that the present work was started. Only the factor of nutrition has been considered so far, with special attention being given to its effect on

body weight, skeletal growth, reproduction, milk production, and general physical health and development. Experiments are under way at this station to determine the effect of other factors such as temperature and humidity, age at first calving, heredity, and certain physiological factors such as the ductless glands.

GENERAL PLAN OF THE EXPERIMENT

Three groups of calves were used. The first group was weaned between thirty and forty days of age, and placed on a dry grain and hay ration. The second group was handled in a manner similar to group 1, but the calves were weaned at forty to fifty days of age. The third group was weaned at thirty to forty days of age as were the animals in group 1, but blackstrap molasses was added to the ration during the greater part of the feeding trial.

REVIEW OF LITERATURE

The study of the growth of dairy heifers has attracted considerable attention. Though only a few of the investigations have a direct bearing on the work reported here. Fohrman (3) weaned calves at sixty to seventy days of age and then placed them on a dry grain and hay ration. These calves grew and developed into normal, healthy animals. One of us (4) in making a similar study, weaned calves at sixty to seventy days of age. These animals also developed into normal animals. In both of these trials it was found that while growth, as measured by weight, was in some cases quite seriously checked shortly after the weaning period, the growth of the skeleton continued at a nearly normal rate. After a period of two to three months following the weaning period the animals made abnormal gains in weight. Waters (5) has shown that the body weight is much more easily influenced than the skeletal growth by the conditions to which the animal is subjected. Animals may continue to grow in skeleton and at the same time remain at a constant weight. It was shown that the tendency to recover was strong, following a period of adverse conditions. Eckles (6) studying the effects of the liberality of the ration, concluded that,

there was a strong tendency for animals to recover from retarded growth when conditions are favorable later. This may be accomplished by a more rapid rate of growth, or by prolonging the period of growth. If the retardation, especially in skeletal growth, has gone too far, the animals will not, however, reach the normal size.

There is conclusive proof, therefore, that an animal may be temporarily stunted, and yet reach the normal size for the breed if placed under favorable conditions later. The extent to which an animal may be stunted and yet reach the normal is not so well known. This would seem to be a point worthy of investigation.

That calves can not be raised on milk alone has been shown by Davenport (7). A calf which received only milk developed swelling of the joints and disturbances of the nerve centers. As soon as hay and straw were allowed, the calf improved rapidly. As much as one half a bushel of grain daily, to a five months old calf, did not satisfy its craving for food. It is interesting to note that this enormous consumption of grain did not cause any digestive disturbances. McCandlish (8) has also shown that milk alone will not supply all the nutritional wants of calves after they are a few weeks old. He concludes that "the lack of bulk may arrest development of the alimentary tract and prevent proper digestion of the nutrients supplied by the milk." These calves grew fairly well until they were two to three months old, but from this time on they did not thrive though they continued to gain slowly in weight for another thirty days; after which their live weight decreased gradually until the time of death. The body measurements appeared to increase about normally until the time the live weight increase ceased to be rapid, and from this time on the measurements were almost constant.

Feeding experiments in which dry grain and hay were used as the sole source of nourishment at as early an age as reported in this paper, do not appear in the literature so far as the authors could find. Reports of experiments where milk feeding has been supplemented by grain and hay are quite numerous, even at an age far below that at which milk alone has been found to be

inadequate. In most of these experiments corn, bran, oats, and alfalfa or clover hay, have played an important part. The reason for the good results obtained from these feeds is just becoming known. Steenbock and Boutwell (9) have shown that yellow corn is a more valuable source of the fat-soluble vitamine than white corn, and Steenbock and Gross (10) have shown that alfalfa contains the fat-soluble vitamine in comparatively large amounts. It is also a valuable source of mineral as shown by Hart, Steenbock and Fuller (11) and its proteins appear to be of good quality. While oil meal probably contains only a slight amount of the fat-soluble vitamine, it is high in protein, and its laxative nature has an important bearing on the physiological value of the ration. Wheat bran contains considerable phosphorus, lends bulk to the ration, and contains some of the water-soluble B vitamine. Osborne and Mendel (12) found the crude protein of wheat bran to have a higher value for the growing animal than the embryo, but according to Forbes and Beigle (13) wheat bran is unsatisfactory as a source of calcium. However, Hart, Steenbock and Fuller (14) state that ruminants consuming the usual roughage will ordinarily receive calcium enough for growth. In view of these findings the following grains were used in making up the rations used in the work reported here; yellow corn meal, old process oil meal, wheat bran and alfalfa meal. The alfalfa meal was used in place of the hay only for convenience in weighing.

With the idea of increasing the palatability of the ration, blackstrap molasses was added for a short period of time. Calloway (15) in studying the physical effect of low grade sugar cane molasses when fed to young calves, concluded that, "when calves begin to consume a little grain, they can be given as much as one to two ounces of blackstrap molasses with each feed with perfect safety." He states that the molasses should be increased gradually. The calves were started on molasses at an average age of four weeks. In the case of the calves reported here, the feeding of molasses caused scours in some cases while in others no harmful effects were noticed. This may be accounted for by the fact that there was no satisfactory method of mixing the molasses

with the grain or alfalfa meal; and as a result certain of the calves picked out the lumps of grain or alfalfa containing the molasses and in so doing received a higher percentage of molasses than would otherwise have been the case.

A great deal has been said regarding the use of minerals in the ration of dairy animals. In this study no minerals were used during the early part of the feeding trial. It was felt that the grain mixture and the alfalfa meal would supply the required minerals. It was noticed, however, that certain calves were eating dirt and licking the fence around the exercise yard, and for that reason calcium carbonate and raw rock phosphate were added to the grain mixture to see if such an addition would have any effect on the calves. Later, these minerals were placed in separate boxes accessible to the calves at all times. This amount was weighed when put into the boxes and at the end of ten days was weighed back. This was done with the view of determining whether or not the calves would express any special need for one mineral more than the other.

METHOD OF PROCEDURE

Animals used[2]

All pure bred female calves dropped in the Experiment Station herd during the period the experiment were used. They were not selected. It was not possible, therefore, to have an equal number of representatives of each breed. The letter following the number of each calf indicates the breed. As follows: a means Ayrshire, h, Holstein, j, Jersey, and s, Shorthorn.

Rations used and method of feeding

The calves were left with the dams for forty-eight hours and were then placed in individual pens where they were fed milk three times daily; the amount varying with the individual according to appetite and condition of bowels. The calves were

[2] The Experiment Station herd of Jerseys was sold to the University of California and for this reason the Jerseys used in this experiment were not kept on the experiment as long as were the animals of the other breeds.

allowed to run together between feedings. At an average age of four weeks, the calves were given a grain mixture and alfalfa meal. These feeds were placed in the individual feeding pens in separate containers at the time of the night feeding of milk. These containers were so arranged as to prevent the calf from tipping them over. At the end of the milk feeding period the grain and hay were placed in the containers at 5:00 p.m. and left there until morning, when the calves were turned out together in the community pen.

All milk, grain and alfalfa meal were carefully weighed, and any grain or meal left by the calves was weighed back and this amount deducted from the original amount. As soon as the calves reached one year of age, they were given the grain mixture which is used at this station for all growing dairy animals, and were also allowed all the alfalfa hay they would eat in place of alfalfa meal. They also received a small amount of corn silage. The hay and silage were not weighed. During the pasture season all calves over a year of age were turned out to pasture. The grain mixture consisted of 6 parts each of hominy and wheat bran, 4 parts each of old process oil meal and ground oats, and 2 per cent each of salt and raw rock phosphate.

The calves were watered twice daily. They did not have water while in the individual pens, hence did not drink while they were eating their dry grain and alfalfa meal. The calves were weaned gradually, ten days being allowed for the change from milk to the dry ration. The bedding consisted of rye straw.

Measurements

Eckels (16) in a study to determine the best methods of measuring the growth of dairy heifers, concluded that the growth of an animal could not be properly represented either by live weight or any body measurements alone. It was decided that the most satisfactory plan is to use both the live weight and some body measurement which represents skeletal growth, and for the latter it was concluded that height at withers is the most satisfactory. In this study, the calves were weighed every ten days, until they were one year of age. Weights were taken on the ninth, tenth

and eleventh days of each period, and the average of these three days was used as the weight for the tenth day. As soon as the calves reached one year of age, they were weighed every thirty days.

The height at withers and heart girth measurements were taken every thirty days from birth. The last named measurement was taken with the view of obtaining additional data on the growth of the skeleton. No check animals were used. All weight and height measurements were compared with the normal growth figures of each breed, established by Eckles (17). The curves shown, represent the percentage of the normal weight and height.

EXPERIMENTAL WORK

Group 1

The animals in this group consisted of 3 Jerseys, 2 Holsteins and 2 Ayrshires. They were allowed milk as previously described until they reached an average of thirty days of age; at which time they were gradually weaned until at forty days they were receiving only dry grain and alfalfa meal. The grain mixture during the early part of the trial consisted of 4 parts of yellow corn meal, 3 parts of old process oil meal and 1 part of wheat bran, and 2 per cent of salt. It was noticed that the calves had a tendency to scour so 2 parts of oil meal were used in place of 3 parts. These proportions of grains were used during the remainder of the trial. Calf 1j received the first mixture until she was 220 days of age; calves 2j and 3j until 185 days of age, and calves 4h, 5h, 6a, and 7a, until 130 days of age.

The calves were limited to 5 pounds of grain per day; no limit was placed on the alfalfa meal. An effort was made to induce the calves to consume as much grain as possible up to 5 pounds and also as much alfalfa meal. For this reason the amount placed in the feed containers was increased each day until it was found the calves were leaving some; the amount was then reduced slightly and increased again as soon as possible.

As previously mentioned the calves showed a tendency to eat a slight amount of dirt which was believed to indicate a lack of

minerals. To all outward appearances, however, the calves were healthy except that they were rather thin and did not show the finish of milk fed calves. Two per cent of raw rock phosphate and 2 per cent of raw limestone were added to the grain ration when the calves were at the following ages: Calf 1j, 240 days, calves 2j and 3j at 200 days and calves 4h, 5h, 6a, and 7a at 150 days of age. At the end of thirty days it was decided to leave the minerals out of the grain mixture and to place two boxes in the community pen, one containing raw rock phosphate and the other, raw limestone. As the experiment progressed and the animals which constituted groups 2 and 3 were added they also were turned out during the day in the community pen and had access to the mineral boxes. Ten pounds of the minerals were placed in the separate boxes and at ten-day intervals the amount remaining was weighed and in this way an approximate figure was obtained as the amount consumed by the calves. At the end of the month the total was divided by the number of calves running in the pens during that month. As can be seen this was not a very accurate method but it served the purpose in that it gave an opportunity to observe whether or not the calves would eat more of one mineral than another and also if the amount consumed would stop the calves from eating dirt. As shown by table 1, which gives the average amounts of the minerals consumed per calf per month, the amount decreased each month until it was almost negligible. The calves however did not show any outward effect from the use of the minerals. As before, certain individuals were noticed to eat dirt in very slight amounts. Apparently this was not due to any lack of mineral unless the minerals in the form fed were not utilized by the animals. However, it was decided to add 2 per cent of raw rock phosphate to the grain ration after the box method of supplying the minerals was discontinued.

Calves 6a and 7a were born only two days apart and as was the case with practically all of the Ayrshires, did not grow as well as the Holsteins. Neither did these two consume as much grain or alfalfa meal as did the other calves in this group. For this reason an effort was made to increase the palatability of the ration by

adding molasses with the view of inducing calf 6a to consume larger quantities of the feed. At 220 days of age 40 per cent of molasses was added to the alfalfa meal given to this calf and 10 per cent was added to the grain ration. She was continued on this ration until 330 days of age and in that time consumed 451 pounds of grain and 308.8 pounds of alfalfa, while calf 7a consumed 469 pounds of grain and 276 pounds of alfalfa. During this time calf 6a gained 145.7 pounds in weight, and calf 7a gained 130.8 pounds in weight. As can be seen there was very little difference in the amounts of grain and alfalfa consumed by

TABLE 1

Average consumption of minerals per calf per month

MONTH	RAW LIMESTONE	RAW ROCK PHOSPHATE
	pounds	*pounds*
1	0.760	0.31
2	0.446	0.53
3		0.153
4		0.362
5		0.000
6		0.270
7		0.204
8		0.000
9		0.057
10		0.028
11		0.028

the two calves and the gains made by calf 6a were only slightly better than those made by calf 7a.

In figure 1 are the curves which represent the percentage of the normal weight and height for these calves.

Table 2 gives the total gains in weight, height and heart girth measurements and total feed consumed from birth to 300 days of age for each animal in the three groups.

Group 2

The calves in this group consisted of 3 Jerseys, 2 Ayrshires, 2 Shorthorns and 1 Holstein. They were fed and handled in a manner similar to the calves in group 1 except that the weaning

period began at forty days of age instead of thirty. The grain mixture used for this group consisted of 4 parts of yellow corn meal, 2 parts of old process oil meal and 1 part of wheat bran and 2 per cent of salt. After the box method of feeding the minerals was discontinued as described under group 1, these calves received raw rock phosphate in their grain ration at the rate of 2 per cent of the weight of the total grain mixture. In figure 2 are the curves representing the average percentage normal weight and height for each breed represented in group 2.

Group 3

The animals in this group consisted of 6 Holsteins, 3 Jerseys, 2 Ayrshires and 1 Shorthorn. They were fed and handled in a manner similar to the calves in group 1 except that 10 per cent of blackstrap molasses was added to the grain mixture and 20 per cent to the alfalfa meal. Two per cent of raw rock phosphate was added to the grain mixture.

Two or three times it was necessary to leave the molasses out of the ration for a day or two as it appeared to cause scours. It may be that other factors were at work although it is believed the molasses was the cause since the calves in group 1 did not scour except during the period when the ration consisted of 3 parts of oil meal in place of 2. As previously explained the inefficient method of mixing the molasses with the grain and hay may have had something to do with the calves scouring. Through a more thorough mixing of the molasses in the ration the calves would not have received so high a percentage of the molasses.

DISCUSSION OF RESULTS

The calves in all three groups were surprisingly healthy during the greater part of the feeding trial. Scouring occurred only in the case of a few individuals and then only during the milk feeding periods and in the case of the calves in group 1 at the time when 3 parts of oil meal were used in place of 2 parts. A few of the calves in group 3 scoured somewhat at various intervals. This was believed to be due to the molasses. Collectively there was less trouble with scours than is usually experienced with milk fed calves.

TABLE 2

Total feed consumed and increase in weight, height and heart girth* from birth to 300 days of age*

CALF NUMBER	WEIGHT	HEIGHT	HEART GIRTH	MILK	GRAIN	HAY
Group 1						
	pounds	*cm.*	*cm.*	*pounds*	*pounds*	*pounds*
1j	392.6	29.3	53.1	342.0	1,060.5	735.5
2j	315.3	32.0	56.0	222.0	1,025.0	612.5
3j	325 4	28 4	52 8	183 0	1,153 0	628 0
4h	417.3	28.4	57.6	281.0	1,210.0	851.5
5h	484.4	36.5	59.5	429 5	1,199 5	1,464 2
6a	205.7	18.1	37.9	418.0	740.0	389.3
7a	224.7	16.8	38.3	414.0	711.7	481.5

Calf 2j—figures for height and heart girth are from forty days to three hundred days of age.

Calf 4h—figures for height and heart girth are from sixty days to three hundred days of age.

CALF NUMBER	WEIGHT	HEIGHT	HEART GIRTH	MILK	GRAIN	HAY
Group 2						
8a	371.1	26.9	52.5	415.0	829 0	950.0
9a	325.0	23.5	52.8	387.5	930.5	599.0
10s	250.2	22.5	50.5	429.5	778.5	298.5
11s	297.9	25.5	51.1	350.0	973.0	450.0
12h	375.0	29.8	52.0	508.5	790.0	496.0
13j	317.7	29.5	55 0	361 0	1,014.0	801.0
14j	246.6	25.5	40.0	355.5	934.0	611.0
15j	332.0	29.7	53.1	392.5	995.0	678.0

Calf 9a—figures for feed are from birth to two hundred sixty days of age.

Calf 12h—figures for feed are from birth to two hundred thirty days of age.

Calf 14j—figures for weight are from ten days to three hundred days of age.

CALF NUMBER	WEIGHT	HEIGHT	HEART GIRTH	MILK	GRAIN	HAY
Group 3						
16h	401.6	35.4	49.6	374.5	1,150.0	1,069.8
17h	394.6	28.1	45.3	363.5	1,097.0	964.0
18h	366.6	32.3	51 6	318.0	1,046.0	955.0
19h	320.0	24.7	42.4	263.5	934.0	502.5
20h	152.3	18.5	28.3	329.5	497.5	304.5
21h	343.3	24.9	50.9	347.5	937.7	783.5
23j	299.4	31.1	46.1	338.0	1,099.0	682.0
24j	168.6	19.4	27.9	281.0	832.0	263.0
26a	169.7	16.0	32.7	201.0	443.1	302.0
27a	236.3	20.2	38.5	197 0	737 0	365.5
28s	255.5	23.8	40.6	132.5	883.0	313.0

Calf 20h—figures are from birth to two hundred fifty days.

Calf 21h—figures are from birth to two hundred ninety days.

Calf 24j—figures are from ten days to two hundred seventy days of age.

Calf 27a—figures are from ten days to three hundred days of age.

* Increase in height and heart girth is calculated from thirty days of age to the measurement taken nearest to three hundred days of age as shown on the individual tables of growth by thirty-day periods.

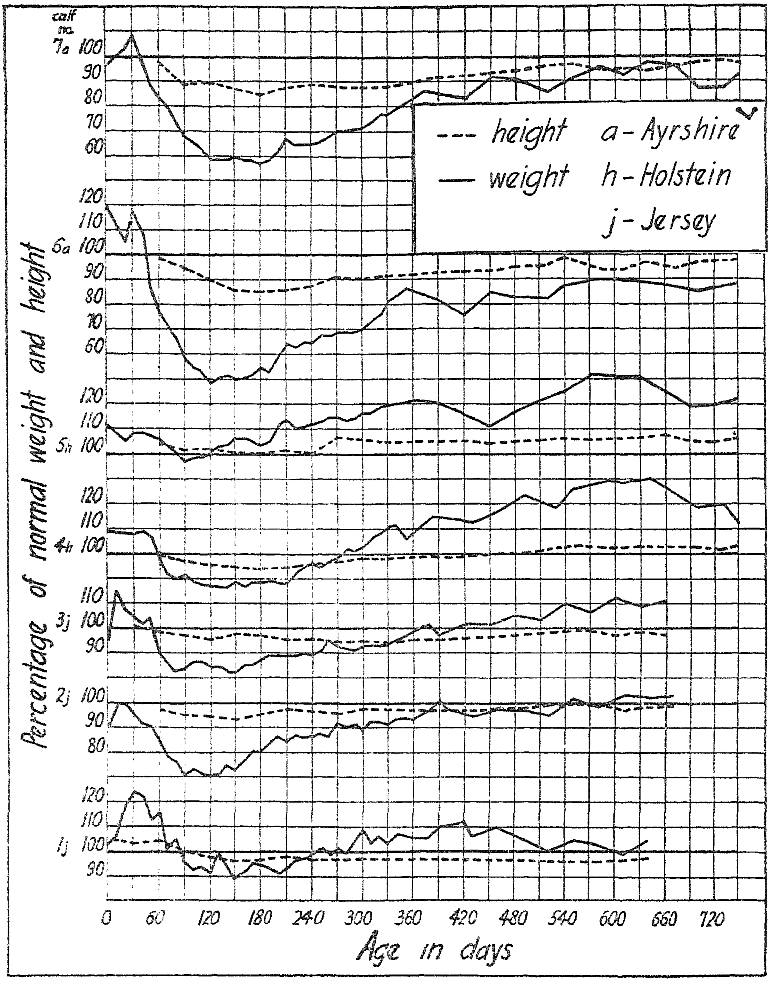

FIG. 1. GROUP 1, REPRESENTING THE PERCENTAGE OF THE NORMAL WEIGHT AND HEIGHT AT WITHERS

The calves in this group were weaned between thirty and forty days of age and were then placed on a dry grain and hay ration.

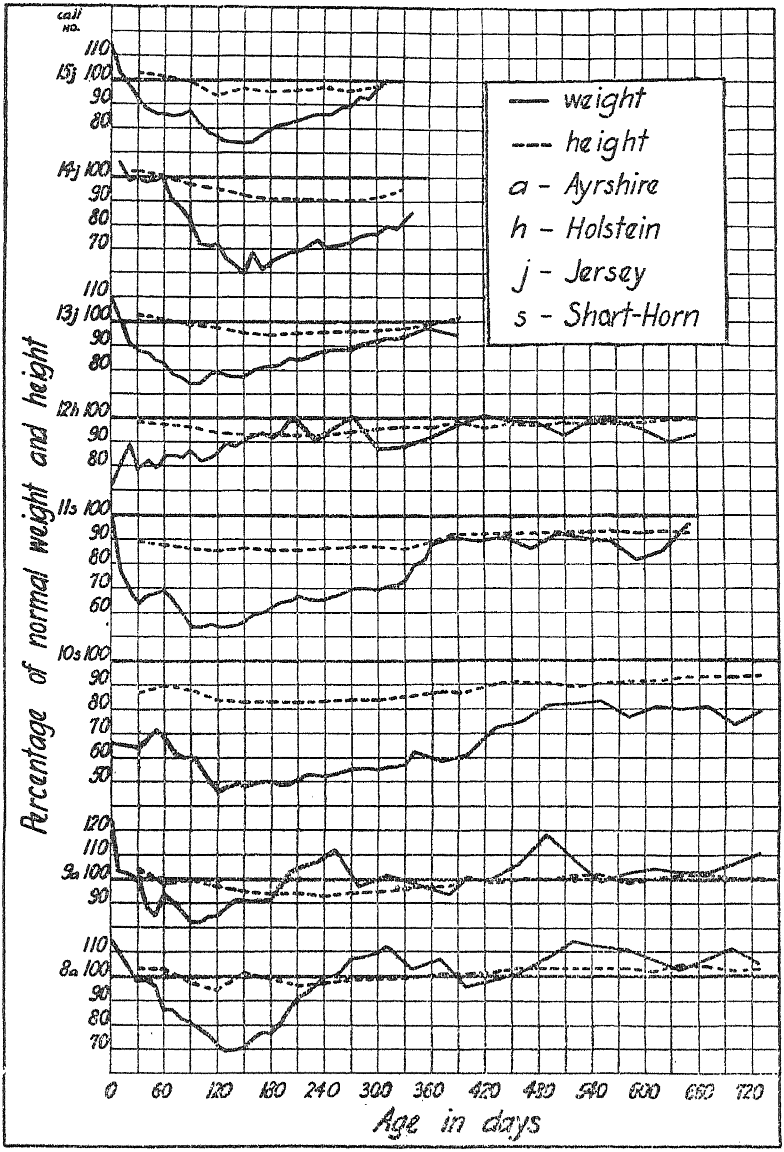

FIG. 2. GROUP 2, REPRESENTING THE PERCENTAGE OF THE NORMAL WEIGHT AND HEIGHT AT WITHERS

The calves in this group were weaned between forty and fifty days of age and were then placed on a dry grain and hay ration.

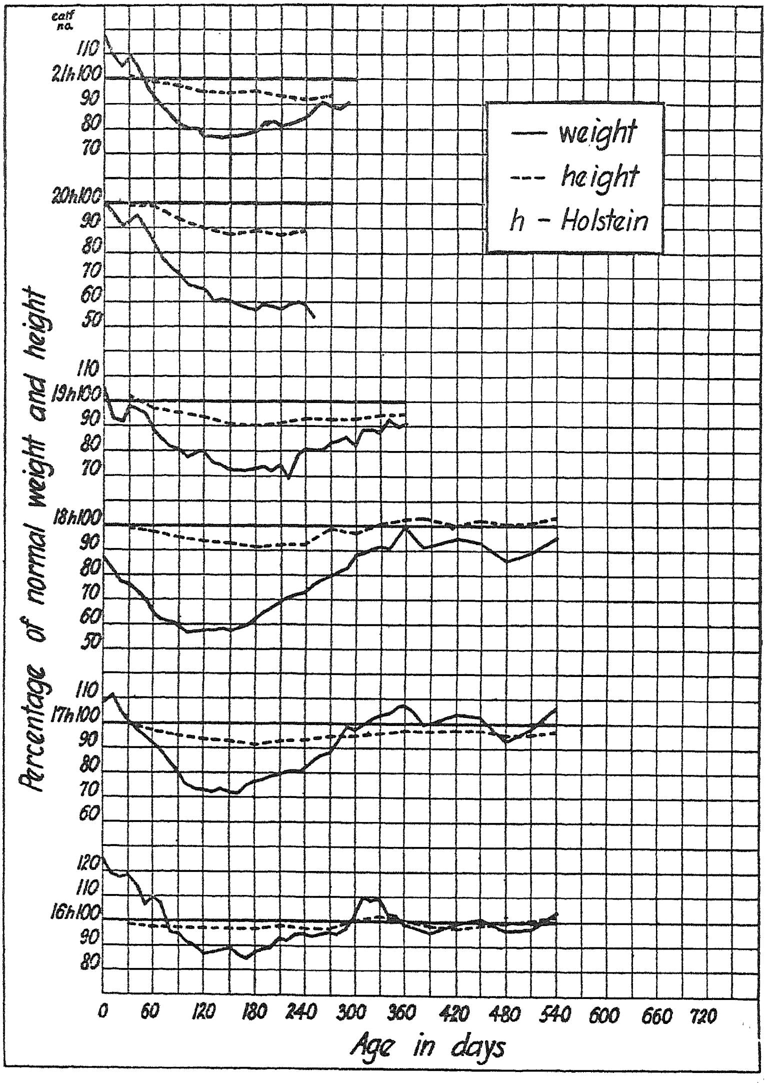

FIG. 3. PART I. REPRESENTING THE PERCENTAGE OF THE NORMAL WEIGHT AND HEIGHT AT WITHERS

The calves in this group were weaned between thirty and forty days of age and were then placed on a dry grain and hay ration. The ration received by these calves differed from that received by the calves in groups 1 and 2 in that 10 per cent of molasses was added to the grain mixture and 20 per cent to the alfalfa meal.

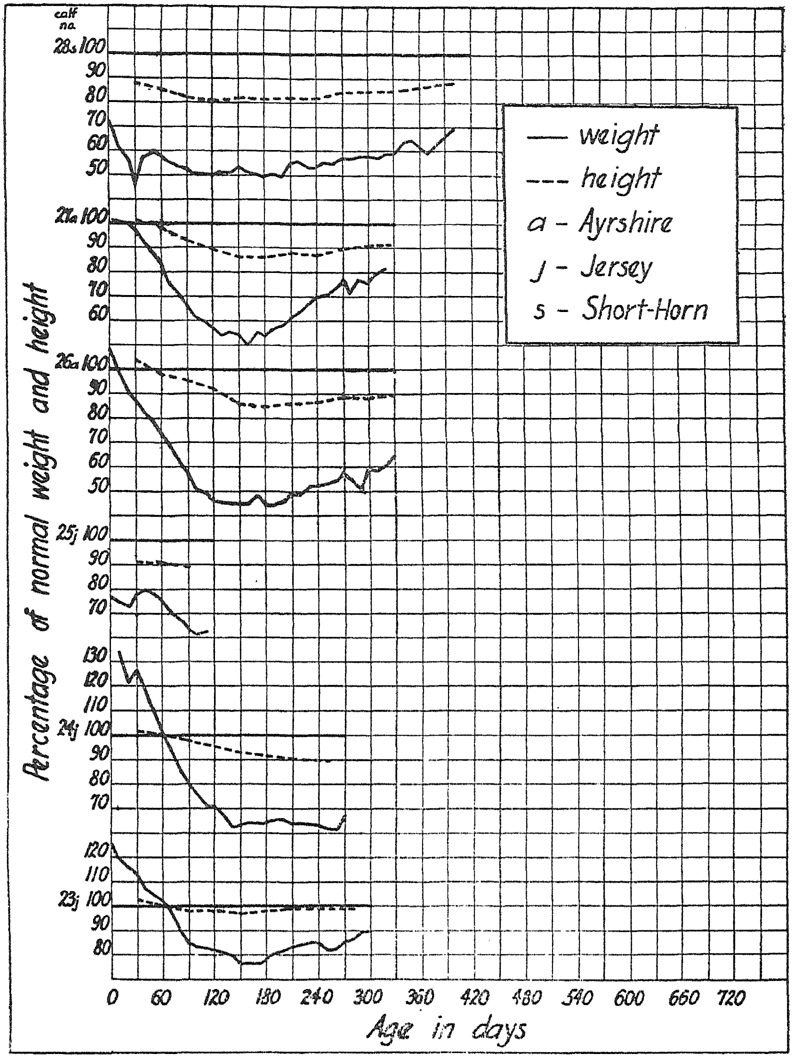

FIG. 3. PART II. REPRESENTING THE PERCENTAGE OF THE NORMAL WEIGHT AND HEIGHT AT WITHERS

The calves in this group were weaned between thirty and forty days of age and were then placed on a dry grain and hay ration. The ration received by these calves differed from that received by the calves in groups 1 and 2 in that 10 per cent of molasses was added to the grain mixture and 20 per cent to the alfalfa meal.

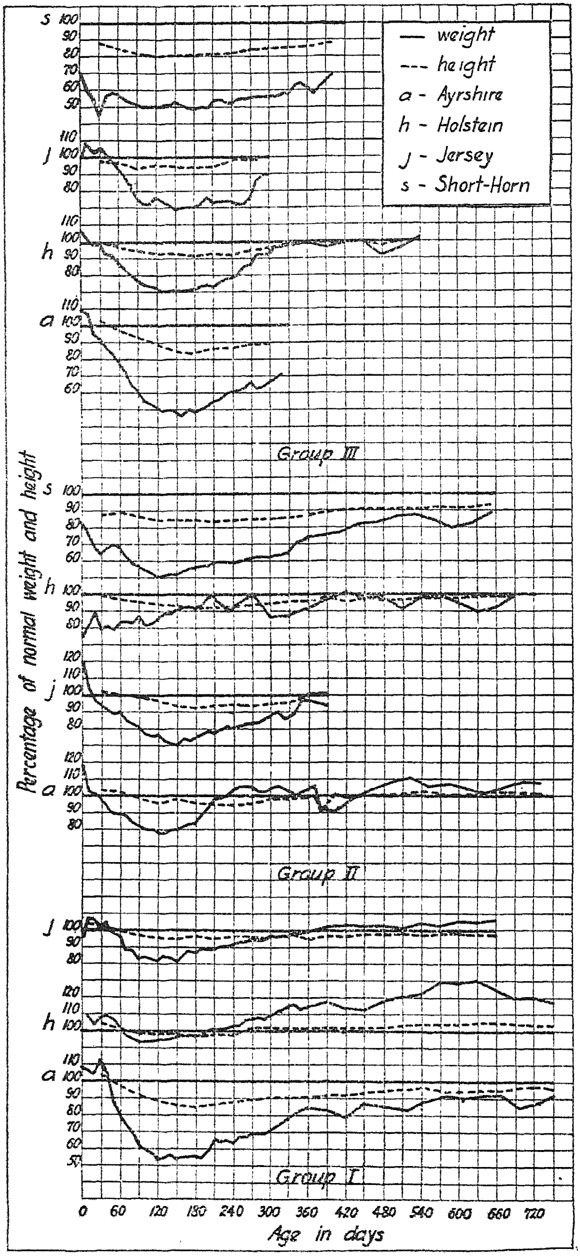

Fig. 4. Representing the Percentage of the Normal Average Weight and Height at Withers of the Breeds Represented in Each of the Three Groups of Calves

The pot-bellied condition so common to calves fed on so-called milk substitutes was absent in the case of the calves reported here. They did not carry the same amount of flesh and were not as slick as milk fed calves and shortly after they were weaned most of the calves dropped considerably below normal in weight, and in some cases their skeletal growth was also considerably retarded, but not to the same extent as the weight, as shown by figures 1, 2 and 3. The time required to return to the normal was apparently dependent upon the degree to which the growth of the animal was checked after the weaning period. The Holstein calves in all three groups were apparently less affected by the early weaning or were better able to make use of the dry grain and hay ration than were either the Jerseys, Ayrshires, or Shorthorns.

The results obtained are in no way conclusive especially in regard to breed characteristics, since only a small number of representatives of each breed were used. It is interesting to note, however, the variation in results obtained from the different breeds. In group 1, the Holsteins returned to normal weight at an average age of 200 days, the Jerseys at 360 days, and the Ayrshires were practically normal at 760 days of age. In group 2, the calves were weaned between 40 and 50 days of age, in this case being allowed milk ten days longer than were the calves in group 1. There was only 1 Holstein in this group so the error due to the individuality may be considerable. This Holstein returned to normal at 270 days of age but fell slightly below normal a little later, possibly due to the fact that it was turned out to pasture at the age of 230 days. The 2 Ayrshires returned to normal at an average age of 230 days. The 3 Jerseys were not quite normal at 390 days of age. The 2 Shorthorns were still 10 per cent below normal at 650 days of age. The calves in group 3 were weaned at the same age as were those in group 1, but received molasses in their grain and hay. It is not so easy to compare the results in this group with those in groups 1 and 2, since the calves at the time of this report were not as old as those in groups 1 and 2. However, the percentages of the normal for the Jerseys in both groups at the same age were practically the

same as was also the case with the Ayrshires. There were no Shorthorns in group 1, hence there is no basis for comparison. In group 3, the Shorthorn calf was still considerably below normal at 400 days of age. Comparing it with the Shorthorns in group 2, at the same age, there was a difference of 6.6 per cent in favor of the Shorthorns in group 2 who were weaned ten days later than those in group 3. There were 6 Holsteins in group 3, 4 of which had reached 360 days of age at the time of this report. Of these 4 one had practically reached normal at 290 days of age, one at 300 days, one at 360 days, and one was 93.6 per cent normal at 340 days. Of the others, one was only 54.8 per cent normal at 250 days and the sixth calf was 91.4 per cent normal at 290 days of age. For the most part they compare quite favorably with the Holsteins in group 1.

In making these comparisons the height at withers and heart girth measurements are not included since the skeletal growth was normal in most cases at an earlier age than was the growth in body weight. As shown by Eckles (18) and Waters (19) the body weight was much more easily influenced than the skeletal growth by the conditions to which the animals were subjected. The Ayrshires and Shorthorns were the only animals whose skeletal growth showed any great abnormality at any time and in these cases the height at withers approached normal at an earlier age than did the body weight; although in many cases after the weight had returned to normal the height at withers still remained slightly below normal as shown in figures 1, 2 and 3.

This may mean that the skeleton was slower to recover from retarded growth than the body weight or it may be that individuality was the limiting factor.

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THE IMPROVEMENT OF FLAVOR AND KEEPING QUALITY OF HAND-SEPARATOR-CREAM-BUTTER

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When pasteurization of cream for buttermaking was first introduced into the creamery, it was for the sole purpose of making the resulting butter a safer product. It was soon discovered however that pasteurization also influenced the flavor to some extent, thus it became less pronounced but of a more delicate character. Originally the same temperatures were employed for pasteurization of cream for buttermaking as for market milk. It was suggested later by practical creamery operators that somewhat higher temperatures for pasteurization would be beneficial when pasteurizing sour cream since it was claimed that the resulting butter would be of better flavor and that the keeping qualities would be increased. The work herein reported was carried out primarily for the purpose of determining the influence of temperature of pasteurization of sour cream on the flavor of the resulting butter, and the effect of acidity of sour hand separator cream at the time when it is churned.

HISTORICAL

When comparing results obtained by earlier investigators with those published more recently it will be noted that pasteurization of cream for buttermaking is gradually growing in favor. Fifteen or twenty years ago the quality advantages obtained from pasteurization when the resulting products were judged by commercial judges were insignificant if at all noticeable. There were various faults attributed to pasteurization such as overheated flavor, poor texture, etc., and it was held that sour cream could not be pasteurized without too great a loss of butterfat in the

buttermilk. Farrington and Russell (1) determined that sour cream containing over 30 per cent fat might be successfully pasteurized while sour cream containing in the neighborhood of 20 per cent fat would cause a greater fat loss in the buttermilk. Lee (2) found that a slight gain in quality was made as a result of pasteurizing hand separator cream; the gain was rather insignificant while the butter was fresh but was considerably greater after cold storing. It was not considered that pasteurization affected the body or texture of the butter. Curdling of the cream by pasteurization increased the loss of fat in the buttermilk.

Dean (3) found an increased loss of fat in the buttermilk as a consequence of increased acidity in the cream at the time of pasteurization. Mortensen, Gaessler and Cooper (4), in comparing raw cream butter with butter made from pasteurized cream obtained a gain of 0.41 point in favor of the latter when using flash heat at 180°F. and a gain of 2.22 points in favor of the pasteurized cream butter when the holding method of pasteurization was employed. Larson, Fuller, Jones, Gregory and Tolstrup (5), found that butter made from cream pasteurized at 180°F. by flash heat retained its keeping qualities better than butter made from cream pasteurized at 140°F., for 25 minutes or from cream pasteurized at 160°F. for ten minutes.

Several attempts have been made to improve over-ripe cream by partial neutralization. Dean (3) reports that in accordance with his experiments butter scored about 3 points higher after adding milk of lime to the cream before pasteurization and $3\frac{1}{2}$ points higher after adding the milk of lime after pasteurization than when the cream was not neutralized.

Ramsay (6) recommends carbonate or bicarbonate of soda for neutralization, and claimed that the carbon dioxide formed during neutralization is impeded in its passage through the cold mass of cream on account of the viscosity of the latter. This viscosity is lessened when the cream is heated to 170°F., as in pasteurizing and the gas then escapes into the air. As it rises through the mass of cream it carries with it, it is claimed, mechanically the volatile substances which give the cream an unpleasant smell and taste. The result is a product from which unpleasant odors

and taints have been removed. In 1920 Ramsay (7) reported that the mixing of cream and neutralizing agent was more rapid and uniform with the flash method of pasteurization than with the holding method. Wide differences were found in the efficiency of holding machinery in actual use in creameries.

METHODS

Only sour cream was used in the following experiments. The cream was mixed in a cream ripener and divided into two lots which were pasteurized at different temperatures. Comparisons were made between cream heated to 145°F. for thirty minutes and cream heated to 170°F. for twenty minutes, and 180° for twenty minutes. All conditions were as near as possible the same excepting the factor that was being investigated.

Two 10-pound tubs were shipped from each churning to both New York and Chicago. The tubs were numbered consecutively and one of a pair was marked "A" and the other "B." The "A" tubs were scored immediately upon arrival on the market. Both tubs were then placed in cold storage at a temperature of 0°F. After about two months the "A" tubs were rescored while "B" tubs remained in storage for a longer period. Credit is due to the Judges Messrs. P. H. Kieffer, C. W. Fryhoffer, James Rowland and W. N. Mapes, all of New York and Mr. G. W. Bull, Chicago,[1] and to our A. R. Morgan, who assisted us in conducting the work at the college creamery.

Pasteurization at 145°F. vs. pasteurization at 170°F.

Comparing results from seventeen experiments, it was found that eight samples from the cream heated to 170°F. scored the higher while fresh, five samples from the cream heated to 145°C. produced the best result, while both samples scored the same in four experiments. The average score on flavor was 34.6 for butter made from cream pasteurized at 170°F. as against 34.2 for butter made from cream pasteurized at 145°F.

[1] Few of the samples were not taken out of storage for scoring at the proper time, such samples were not considered in our calculations.

After two months storage eleven of nineteen experiments favored the high temperatures while in six of the experiments the lower temperature produced the higher scores and in two experiments the scores were the same. The average score on flavor was 34.5 for butter made from cream pasteurized at 170°F and 33.7 for butter made from cream pasteurized at 145°F.

After nine months storage five of eleven experiments favored the butter made from cream pasteurized at 145°F. Three experiments came out in favor of the higher pasteurizing temperature and in three experiments the results were the same for both methods. The average score on flavor was 32.5 for the butter made from cream pasteurized at 145°F. and 31.8 for butter made from cream pasteurized at 170°F.

Influence of pasteurizing temperature on quality of resulting butter

NATURE OF EXPERIMENT	NUMBER OF EXPERIMENT	AGE OF BUTTER WHEN SCORED	SCORE ON FLAVOR		
			145°F.	170°F.	180°F.
145°F. vs. 170°F.	17	Fresh	34.2	34.6	
	19	2 months	33.7	34.5	
	11	9 months	32.5	31.8	
170°F. vs. 180°F.	9	Fresh		34.4	33.9
	6	2 months		33.2	33.7
	8	9 months		32.6	32.3

Pasteurization at 170°F. vs. pasteurization at 180°F.

Nine experiments were conducted for the purpose of determining if there would be any difference between quality of butter produced from cream pasteurized at 170° and that from cream pasteurized at 180°F. When the butter was fresh, four of the nine experiments came out in favor of a temperature of 170°F. while three were in favor of 180°F. and in two experiments the scores were the same. The average score on flavor for butter made from cream pasteurized at 170°F. was 34.4 and for butter from cream pasteurized at 180°F. 33.9. Butter made from cream pasteurized at 180°F. was often criticized as being over-heated.

After two months storage three of six experiments came out in favor of the higher pasteurizing temperature, one experiment came out in favor of the lower temperature and two were the same for both. The average score on flavor was 33.2 for the butter made from cream pasteurized at 170°F. and 33.7 for cream pasteurized at 180°F.

After nine months storage, four of eight samples came out in favor of the 170°F. temperature, two in favor of 180°F. and two the same for both methods. The average score on flavor was 32.6 for the butter made from cream pasteurized at 170°F. and 32.3 for butter made from cream pasteurized at 180°F.

REDUCTION OF ACIDITY IN SOUR CREAM

Results reported from former experiments conducted at this and other stations seem to indicate that when sweet cream of high quality is received the butter made from cream ripened to a medium acidity possesses the better qualities during the first one or two months but deteriorates faster than butter made from sweet cream. It was therefore our object to determine if the acidity of the cream at the time of churning would be of equal importance when the cream was originally sour and of rather poor quality. It was also the purpose of the following experiments to test out certain methods of manufacture recommended for sour cream. Part of the acidity in the cream was reduced, and for that purpose neutralizing agencies such as lime, viscogen,[2] and bicarbonate of soda (baking soda) were used. The following experiments were carried on in the laboratories in the dairy department in connection with the instructional work.

Conclusive proofs have been presented by this and other stations to the effect that sour cream of poor quality is improved by neutralization, but it has also been held by many buttermakers

[2] Viscogen is a sucrate of lime solution and was made in accordance with Bulletin No. 54, Wisconsin Experiment Station, by dissolving two and one-half parts by weight of a good quality of cane sugar in five parts of water, and one part of quicklime gradually slacked in three parts of water, the two solutions being mixed. The mixture was agitated at frequent intervals and after two or three hours allowed to settle until the clear supernatant fluid could be siphoned off.

that a good starter was of greater importance than neutralization. For the purpose of determining the correctness of that statement the following tests were made.

Butter obtained from partly neutralized cream churned without the addition of starter vs. butter from untreated cream to which starter was added

Cream, usually of high per cent acidity, was mixed in a vat and divided into two lots. One was partly neutralized and pasteurized, no starter being added and the other lot was merely pasteurized and a starter added after the temperature had been brought down to about 60°F. Both lots of cream were cooled to 40°F. and left at that temperature until churning. The greatest possible effort was made to have all other conditions the same for the two lots.

Two 10-ten pound tubs from each churning were shipped to New York or Chicago. They were scored by commercial judges while fresh, and after two months storage. The butter was held in cold storage at about 0°F.

Three out of nine experiments came out in favor of the neutralized cream butter while fresh, five came out in favor of the other while one scored the same for both. The average score on flavor was 33.8 for the neutralized cream butter and 34.4 for the other.

After two months storage the neutralized cream butter scored highest in six of eight experiments, the other scored highest in one and the scores were the same in one experiment. The average score on flavor for the neutralized cream butter was 34.2 against 33.0 for the other.

Neutralized vs. untreated cream butter, starter added to both

Five experiments were conducted to determine results when the same per cent of the same starter was added to each lot. The first scoring was done from one to two months after the butter was made while the second scoring was done after the butter was about nine months old.

At the first scoring, four neutralized cream samples ranked higher against one for the butter made from cream not neutralized.

The average score on flavor for the neutralized cream butter was 33.8 and 32.9 for the butter made from untreated cream.

After nine months four neutralized cream samples ranked higher against one for the butter made from untreated cream. The average score on flavor was 32.6 for the neutralized cream butter against 30.2 for the untreated cream butter.

Pasteurization experiments with neutralized cream

NATURE OF EXPERIMENT	NUMBER OF EXPERIMENT	AGE OF BUTTER WHEN SCORED	SCORE ON FLAVOR			
			Neutralized without starter	Untreated with starter	Neutralized with starter	Ripened neutralized cream
Neutralized cream without starter vs. untreated cream with starter	9	Fresh	33.8	34.4		
	8	2 months	34.2	33		
Neutralized vs. untreated-cream butter. Starter added to both	5	Fresh		32.9	33.8	
	5	9 months		30.2	32.6	

DISCUSSION OF RESULTS

From the above data, it seems fair to conclude that sour cream pasteurized at 170°F. by the holding method produces a slightly superior product than is produced from the same cream when it is heated to only 145°F. It seems however that butter made from cream pasteurized to a high temperature does not keep as well over a long period of time as butter made from cream heated to a lower temperature. It may be possible that the exposure of the fat to a higher heat hastens oxidation. Pasteurization at 180°F. for twenty minutes did not give as satisfactory results at 170°F., the resulting butter often being criticized for having an overheated flavor.

It seems to hold true when handling sour cream for buttermaking that the flavor of the product is improved and the resulting butter deteriorates about in proportion to the per cent of acid contained in the cream at the time of churning. For these reasons some of the creameries are today neutralizing part of the

acidity in sour cream before churning. This practice is undoubtedly to the advantage of producers who live on cheap land in territories where dairying has not been developed and for that reason have to ship their cream long distances. This system should however not be encouraged in the better dairy districts where the producers live within few miles of a creamery as in that case it has a tendency to cause the producers as well as creamery operators to become somewhat indifferent about quality of the raw material. The dairyman who is farming rather high priced land will obtain a profit from his dairy herd only when he is producing a product which is of high quality.

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THE RELATION OF NATURAL ACIDITY IN MILK TO COMPOSITION AND PHYSICAL PROPERTIES

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The reaction of milk has long been a subject of scientific, as well as practical, interest. On standing under ordinary conditions milk will become sour due to the formation of lactic acid from bacterial action. But aside from this, the milk of most animals is slightly acid when drawn. This natural acidity varies from one individual to another, and, even from one quarter of the udder to another in the same individual. It is the purpose of this work to find what constituents in milk cause these differences in acidity, how the properties of milk are affected by these factors, and to contribute data which may add to the general knowledge of the intricate composition of milk.

In some of the earliest work, investigators reported that milk would change blue litmus paper to red and red to blue (1). To describe this condition the term "amphoteric" was applied. It is hardly conceivable to us now how this could be true. It is likely, however, that if litmus paper be left in contact with some kinds of milk sufficiently long that the dye would be adsorbed by the colloids of the milk, and what was really a fading out of the paper was described as a color change.

A little later when other indicators than litmus were employed, there seemed to be some justificatoin of the idea. Thomsen (2) found that milk was acid to phenol phthalein and alkaline to lacmoid. Of course, we see it in our day as a demonstration of the fact that the hydrogen ion concentration of average milk lies between the points at which these indicators change colors.

Quantitative determination of acidity in milk began with Soxhlet and Henkel (3) who titrated with standard alkali using phenol phthalein as indicator. That original procedure has not

been changed to the present time except in the method of expressing the results and in the nature of alkali used.

Measurement of concentration of hydrogen ion in milk was first carried out by Foa (4) and a large number of measurements made since that time have shown the average for cow's milk to be about pH 6.5, with variations from 6.3 to 7.2.

Though some determinations of pH were made in this work, particular attention was given to acidity by titration. It is believed that results obtained by this method are more useful in judging the condition of milk, as well as changes taking place in it. The very nature of milk with its high content of buffer substances is such that the pH tends to remain constant regardless of additions or changes. Unless otherwise stated, the term "acidity" in this paper will refer to acidity of titration.

The majority of writers have believed acidity in milk to be due to one or all of the following substances: monobasic phosphates, casein, acid citrates and carbon dioxide. Lactic acid is known not to be present in freshly drawn milk but the acidity is generally calculated in terms of the percentage of lactic acid for the sake of convenience.

The milk of individual cows has for a long time been known to vary in acidity. Some factors which influence acidity as well as the composition of milk are—health of cow, age, breed, feed, period of lactation, differences in morning and evening milk, different portions of the same milking, and different quarters of the udder. In this work no effort was made to collect data regarding physiological causes for variations in acidity. Samples of milk from a large number of cows were titrated and those were selected for study which showed highest and lowest values, together with various points between, regardless of their sources; all animals were, however, fed similarly, and all were apparently in good health. On those chosen, a large number of analyses were first made the results of which are given in table 1.

Acidity was determined by titrating 20 cc. of milk, undiluted, with 0.1 N sodium hydroxide using 1 cc. of neutralized phenol phthalein solution (5 grams in 1 liter 50 per cent alcohol), and calculating results in terms of percentage lactic acid.

TABLE 1

COW NUMBER	PER CENT ACIDITY	SPECIFIC GRAVITY	PER CENT TOTAL SOLIDS	PER CENT FAT	PER CENT SOLIDS NOT FAT	PER CENT LACTOSE	PER CENT TOTAL PROTEIN	PER CENT CASEIN	PER CENT ALBUMIN	PER CENT BY VOLUME		PER CENT CITRIC ACID	PER CENT ASH	ALKALINITY OF ASH	PER CENT P_2O_5	PER CENT CaO
										Total CO_2	Free CO_2					
3	0.225	1.0301	13.34	3.90	9.44	5.05	3.30	2.49	0.731	8.00	4.07	0.191	0.733	60.2	0.2719	0.1631
19BF*	0.207	1.0331	12.31			5.15	3.33	2.65	0.675				0.810		0.2826	0.2125
20	0.202	1.0290	13.56			5.23	2.91			7.13	3.92	0.142	0.743	52.7	0.2534	0.1568
24	0.194	1.0319	13.65			4.73	3.80						0.811		0.2849	
21	0.192	1.0280	12.09			5.65	2.94						0.737		0.2277	
16	0.157	1.0282	11.72	2.60	9.12	4.87	2.92					0.199	0.709	57.2	0.2025	0.1450
Herd	0.153	1.0296	12.34	3.80	8.54	4.88	2.98			9.93	4.18		0.729	56.4	0.2156	0.1515
22	0.126	1.0261	11.69			4.15	3.06						0.800		0.2148	
23	0.121	1.0277				4.80	2.77	2.14	0.626	9.42	4.63	0.180	0.715	52.5	0.1753	0.1527
18	0.112	1.0270	11.30	3.40	7.90	4.35	2.85			14.10	5.45	0.116	0.771	54.7	0.1660	
19LH†	0.108					3.25	2.82	1.70	1.114				0.822		0 1962	0.1511

* Both front udders of cow 19.

† Left hind udder of cow 19.

Determination of total carbon dioxide was made by adding 30 cc. of 20 per cent lactic acid to 300 cc. of milk and passing a stream of carbon dioxide-free air through the boiling liquid into absorption tubes for one hour. "Free carbon dioxide" is that which could be removed from the untreated milk in the cold during a one hour period.

Alkalinity of ash was determined by adding an excess of 0.1 N nitric acid to the ash, boiling for a short time and titrating back with standard alkali using methyl orange as indicator.

Other determinations were made by the usual methods.

With decreasing acidity there is noted a general, though not regular, decrease in specific gravity, total solids, solids-not-fat, lactose, protein, casein, but a somewhat more regular decrease in P_2O_5. Similar observations have been made on some of these points by Hanne (5), Henkel (6), Allemann (7,) and Van Slyke and Baker (8), and others. There seems to be no striking relation between acidity and albumen content, citric acid, ash, alkalinity of ash, nor CaO. Low acid milk certainly has less nutritive value than high acid milk.

More careful attention was next given to those constituents which have been generally believed to contribute to the acidity of milk—carbon dioxide, citrates, casein, and phosphates.

RELATION BETWEEN CARBON DIOXIDE AND ACIDITY

Carbon dioxide is a natural constituent of freshly drawn milk and must without doubt affect the acidity to some extent. Taking the values for free carbon dioxide found in table 1, and calculating into terms of its equivalent in percentage lactic acid, the following results are obtained: Cow No. 3—0.016; 19 BF—0.016; herd—0.017; 23—0.018; 18—0.022. Since no particular pains were taken to prevent loss of carbon dioxide from the milk from the time of drawing samples until the determinations were made, these values may be considered as minimum.

Decrease in acidity on boiling or pasteurizing milk can be in part attributed to loss of carbon dioxide. A number of samples were boiled under vacuum at temperatures always below 50°, under which conditions there is little possibility of changes taking

place other than loss of the gases. The decreases in acidity were as follows: Cow No. 3—0.012; 20—0.018; 25—0.014; 16—0.013; herd—0.013; 23—0.018. Values here obtained are seen to be not far from those recorded in the preceding paragraph.

Differences in acidity between the various samples of milk, however, are distinctly not related to carbon dioxide. In fact the milks of low acidity contain somewhat more than those of high. Similar results were reported by Van Slyke and Baker (9). It can only be concluded that a part of the natural acidity is regularly attributable to the content of this gas. Calculated as lactic acid it may amount to 0.01 to 0.02 per cent; though if care be taken to prevent loss of carbon dioxide from time of drawing milk until the determination is made, somewhat higher results should be expected. Also when milk has been stored for a long time or transported long distances the amount of carbon dioxide may be less. These figures can be taken to represent average conditions.

RELATION BETWEEN CITRIC ACID CONTENT AND ACIDITY

It has often been shown that milk contains fairly large amounts of citric acid. This is no doubt combined with more or less base as citrate.

A few solutions were prepared containing citric acid and in one case citric acid with Ca ion present. With the addition of sodium hydroxide these were brought to pH values approximately equal to that of milk. Each solution was titrated to the phenol phthalein endpoint and the acidity calculated as lactic acid. The results are given in table 2.

On the assumption that the condition of the citrates in milk is similar to that in these solutions, it is evident that a part of the acidity of milk is due to this class of salts. However, the effect cannot be large and lies possibly between the values of solutions 3 and 4—in the neighborhood of 0.01 per cent acidity.

Since it was seen that milk of varying acidity did not show corresponding variations in citric acid content, the differences cannot be due to this constituent; its effect must be similar to that of carbon dioxide, rather constant.

RELATION BETWEEN CASEIN AND ACIDITY; EQUILIBRIUM BETWEEN THE VARIOUS CONSTITUENTS OF MILK

While most writers believe that casein is the cause of but a part of the acidity of milk, there are two extreme views: Bordas and Touplain (10) are of the opinion that there are neither free acids nor acid salts in milk, but that all the acidity is due to casein, while Van Slyke and Bosworth (11) conclude that casein is present as neutral calcium caseinate and can exert no influence on the acidity.

It has long been known that the addition of sodium citrate to milk inhibits rennet coagulation. This is commonly explained on the theory that in clot formation calcium ion must be available for combination with the casein and when an excess of citrates are

TABLE 2

SOLUTION NUMBER	PER CENT CITRIC ACID	RATIO—MOLS. NaOH: MOLS. CITRIC ACID	PER CENT CaO AS $CaCl_2$	pH	CC. OF 0.1 N NaOH PER 20 CC. SOLUTION	PER CENT ACIDITY AS LACTIC ACID
1	0.2290	2.75:1	0	6.387	0.6	0.027
2	0.2290	2.84:1	0	6.728	0.4	0.018
3	0.1145	2.84:1	0	6.889	0.3	0.014
4	0.1550	2.92:1	0.05	6.483	0.18	0.008

present unionized calcium citrate is formed thus preventing the combination. Addition of casein to milk has an effect on rennet coagulation similar to that of the alkali citrates (12), the explanation being the same. On the other hand, addition of calcium ion greatly hastens rennet action.

There should be no reason to doubt that for every change in the proportion of the constituents there is a new condition of equilibrium. Casein combined with calcium in amount neutral to phenol phthalein could not remain in contact with a solution of acid salts such as has been found in milk without yielding to those salts some of its base until a condition of equilibrium is established.

A solution was prepared from purified casein and calcium hydroxide, adjusting with acid until just neutral to phenol phthalein, pH = 8.50, also there was prepared a solution containing mono- and di-basic phosphates and alkali citrates with pH = 5.58.

When the two solutions were mixed in equal proportions the resulting pH was 6.49. Base must, therefore, have passed from casein to phosphates to effect this change in pH. As a result the casein must afterward have been acid to phenol phthalein and required alkali to bring it to a point neutral to that indicator. Since the resulting mixture had a hydrogen ion concentration similar to average milk it follows that the casein of milk must be a cause of part of the acidity as determined by titration with phenol phthalein.

Some experiments which apply very strikingly to this hypothesis were reported by Loeb (13). He showed that it requires about 3.5 cc. of 0.1 N alkali to change the pH of a solution of 1 gram of casein from 6.5 to 8.5. This change corresponds to the change in pH when milk is titrated. Using these figures and considering milk to contain 2 to 3 per cent casein, the acidity due to this factor calculated into terms of lactic acid would be 0.063 to 0.095 per cent.

In this work a solution of casein (2.32 per cent) was prepared by adjusting with $Ca(OH)_2$ and weak acid until a pH of 6.66 was attained, and in a similar way sodium caseinate (2.53 per cent casein) with pH 6.66 was made. In former case 1.2 cc. 0.1 N alkali was required to bring 20 cc. to the phenol phthalein end point and in the latter 1.9 cc. Calculated into terms of lactic acid, the former is 0.054 per cent, the latter—0.085 per cent.

On account of the strong buffer action of casein and on account of the complications entering into the equilibrium of milk constituents it is difficult to judge with any great degree of accuracy just what part of the acidity is to be attributed to casein. From the above results, however, it is concluded that 0.05 to 0.08 per cent is due to that factor.

It has already been show that generally milk with high acidity has somewhat larger amounts of casein. It is, therefore, certain that a part of the differences in acidity between various samples is due to this. Yet, as the above calculations show, any possible variations in acidity due to casein cannot be more than a small part of the great differences in acidity actually found in the samples.

RELATION BETWEEN ALBUMIN AND ACIDITY

Loeb found also that it required about 1.5 cc. of 0.1 N sodium hydroxide to change a solution containing 1 gram of egg albumin from pH 6.5 to 8.5. Assuming that lactalbumin reacts in this respect similar to albumin from egg, the titration values for milk containing 0.6 to 0.8 per cent would be 0.008 to 0.011 calculated as per cent lactic acid.

Since no regular relation was found between the percentage of albumin and total acidity this could not be a factor except in contributing approximately a fixed share to the acidity of all milks.

THE RELATION BETWEEN PHOSPHATES AND ACIDITY: A STUDY OF MILK SERUM

As has already been pointed out there was found a distinct variation in P_2O_5 with the acidity. Hanne (14) reports similar observations; Van Slyke and Bosworth (15) conclude that acidity in milk is due exclusively to phosphates.

Serum was prepared from a number of samples of varying acidity. The milk was first skimmed, then passed through a Pasteur Chamberlain filter, the first 75–100 cc. being discarded. In table 3 will be found the acidity of the original milk together with that of the filtrates, and the P_2O_5 content of the latter.

Close comparisons cannot be made between the results obtained on the serum and on the whole milk since the weight relations are not equivalent–100 grams of milk produces much less than 100 grams of serum. Furthermore, as the filter continues to run there is an increasing accumulation of insoluble constituents which possibly shifts the equilibrium and effects the composition of the filtrate. However, it is apparent that practically the entire differences in acidity between the various samples are found in the serum. Since casein is here eliminated from consideration and since the only constituent of importance in the serum that can influence acidity is the phosphate, it must be concluded that differences in natural acidity are due almost entirely to the phosphates.

The actual percentage of phosphorus show much more striking differences in the serum than in the milk. However, no absolute correlation can be made between P_2O_5 and acidity, since the latter depends not only upon the total amount of phosphate but also upon the ratio of monobasic to dibasic salt.

TABLE 3

COW NUMBER	ACIDITY OF MILK	ACIDITY OF SERUM	PER CENT P_2O_5 IN SERUM
20	0.212	0.112	0.1408
24	0.194	0.090	0.1152
21	0.192	0 094	0.1082
Herd	0.153	0.076	0.1000
22	0.126	0.040	0.0852
18	0.112	0.031	0.0622

TABLE 4

COW NUMBER	ACIDITY BY TITRATION	pH	pH:HCl ADDED	pH:NaOH ADDED	DIFFERENCE IN pH:HCl ADDED	DIFFERENCE IN pH:NaOH ADDED
3	0.225	6.432	6.007	6.914	0.425	0.482
20	0.202	6.483	6.024	6.997	0.459	0.514
24	0.194	6.531	6.140	7.024	0 391	0.493
21	0.192	6.495	6.049	7.057	0.446	0.562
16	0.157	6.675	6.149	7.298	0.526	0.623
Herd	0.153	6.690	6.166	7.301	0.524	0.611
22	0.126	6.919	6.381	7.651	0.538	0.732
23	0.121	6.767	6.181	7.501	0.586	0.734
18	0.112	6.898	6.281	7.680	0.607	0.782

HYDROGEN ION CONCENTRATION AND BUFFER ACTION

Hydrogen ion concentration was determined by the Rice and Rider (16) procedure on a number of samples of milk of varying acidity; also, on each sample was determined the pH after addition of 8 cc. of 0.1 N hydrochloric acid to 92 cc. of milk and also with 8 cc. of 0.1 N sodium hydroxide to 92 of cc. milk.

In table 4 will be found the results. It is seen that there is a regular increase in hydrogen ion concentration (decrease in pH values) with increase in acidity by titration, though the former differences cannot be said to be as marked as the latter.

It is shown, also, that the addition of acid and alkali to high acid milk changes the pH much less than when low acid milk is similarly treated. This is to be expected since the proportion of phosphate, particularaly, and of casein is greater in this class of milk; it is the influence of these substances acting as buffers that gives the milk that property.

The fact that high acid milk exhibits a strong buffer action is important in the consideration of the development of lactic acid acidity. For here it is necessary that a larger amount of acid be produced to change the pH than in low acid milk. Differences in pH would not, therefore, be as sensitive a means of determining degree of souring as titration figures.

EFFECT OF DILUTION ON ACIDITY OF MILK

Many investigators have noted that when milk is diluted with water, considerably less alkali is required to bring it to the phenol phtalein endpoint than when the undiluted milk is titrated. This has been attributed by some to hydrolysis of the phosphates (17) and by others to the hydrolysis of the calcuim caseinates (18).

In table 5 is shown the effect of dilution on the titration values of samples of different acidities. In the one case 20 cc. of sample was titrated directly with 0.1 N sodium hydroxide, and in the other, to 20 cc. was added 200 cc. of water before titration. It was found that dilution with more than this amount did not further reduce the acidity appreciably.

It is seen that dilution affects the titration values of high acid milk more than those of low acidity, which would indicate that phosphates have much to do with the effect.

A few samples of serum were titrated similarly, with and without dilution, when it was found that about the same percentage decrease in acidity was produced.

While this shows that constituents other than casein have much to do with the reduction of acidity on dilution, it was found on experimenting with solution of calcium and sodium caseinates already referred to, that there was here also a reduction of acidity on dilution but in somewhat smaller proportion than was found in the milk and serum.

It must be concluded, therefore, that both phosphates and caseinates contribute to the decrease in acidity on dilution.

TABLE 5

SAMPLE NUMBER	ACIDITY. NO DILUTION	ACIDITY. DILUTED 10 TIMES	DIFFERENCE	PER CENT DECREASE
3	0.216	0.149	0.067	31
1	0.185	0.122	0.063	34
26	0.180	0.104	0.076	42
2	0.176	0.117	0.059	33
4	0.168	0.113	0.055	33
17	0.180	0.063	0.045	42
15	0.095	0.054	0.041	43
18	0.086	0.050	0.036	42
Serum 1	0.054	0.036	0.018	33
Serum 2	0.045	0.023	0.022	49
Ca-caseinate	0.050	0.041	0.009	18
Na-caseinate	0.086	0.063	0.023	27

TABLE 6

COW NUMBER	ACIDITY	FREEZING POINT	PER CENT LACTOSE	PER CENT CHLORINE	SPECIFIC CONDUCTIVITY $\times 10^{-4}$ AT 20°
3	0.225	−0.547	5.05	0.0551	41.78
19BF	0.202	−0.553	5 15	0.0634	47.67
20	0.202	−0.546	5.23	0.0583	42.50
24	0.194	−0.552	4.73	0.0896	46.42
21	0.192	−0 550	5 65	0.0743	43.95
16	0.157	−0.547	4.87	0.0890	47.99
Herd	0.153	−0.547	4.88	0.0837	44.42
19BH	0 140	−0.553			61.17
22	0.126	−0.550	4.15	0.1522	60.67
23	0.121	−0.545	4.80	0.1082	49 83
11	0.121				49.00
18	0.112	−0.557	4.35	0.1660	56.70
19LH	0.108		3.25	0.1763	

THE RELATION BETWEEN ACIDITY, OSMOTIC PRESSURE AND CONDUCTIVITY

It has been quite firmly established that the freezing point of milk is its most constant property, and in consequence, can be satisfactorily used as a means of detecting watering (19).

In table 6 it is shown that individual samples of widely varying natural acidity are remarkably constant in freezing point. That

all milks from normal animals of a given species should have the same osmotic pressure and consequently the same freezing point is a theory soundly founded. The crystalloidal constituents are believed to pass from the blood by ultrafiltration (20), and as the blood of all individuals of a species has the same osmotic pressure, so should the milk. Any variation in the amount of one crystalloid in milk is found to be compensated by an opposite variation in others. Lactose and chlorides particularly have been found to thus vary inversely (21). A close inverse relation between lactose and chlorine is noted in table 6, which accounts in large measure for the constancy of freezing point, though, of course, high phosphates in the high acid milk are effective in influencing osmotic pressure in so far as these are soluble.

Some years ago conductivity was believed to be a very constant property of milk but it has since been disproved. The ionizable constituents may vary considerably, and it is these that control the passage of electric current. Since the chlorides, of all the ash constituents, are about the most important in affecting conductivity there should be found some relation between this property and chlorine content. This is, indeed, the case, as is shown in the table.

High acid milk is on the whole richer and contains more lactose. It is roughly true also that it contains less chlorine and has a lower conductivity. The approximate reciprocal relation between lactose and conductivity is also shown.

COAGULATION BY RENNET AND ALCOHOL; ALDEHYDE REDUCTASE

Some investigations have pointed to the fact that there is a relation between the chemistry and colloidal properties of milk and its coagulability with rennet (22), with alcohol (23), and the aldehyde reductase reaction (24).

On samples of milk with different acidities the rennet test was applied by adding to 20 cc., 2 cc. of a weak rennet solution and holding at 35° with gentle agitation. Time necessary for the first appearance of flakes was noted.

The alcohol test was made by determining the least amount of 90 per cent (by volume) alcohol necessary to cause the appearance of flakes when added all at once to 10 cc. of milk.

Aldehyde reductase was measured by adding to 10 cc. of milk 1 cc. of Schardinger's formaldehyde methylene blue reagent (25), covering the surface with a layer of toluene, holding at 47-50°, and noting time of complete disappearance of color.

The results in table 7 show no regular relation between acidity and any of these determinations, though perhaps in general the low acid milks seem to show somewhat greater reductase activity. It should be noted in this connection that Burri and

TABLE 7

COW NUMBER	ACIDITY	RENNET; TIME IN SECONDS	CUBIC CENTIMETERS 90 PER CENT ALCOHOL	REDUCTASE TIME;	
				Minutes	Seconds
3	0.225	42	2.0	9	30
19BF	0.207	102	5.5	4	2
20	0.202	49	2.0	13	15
24	0.194	100	6.5	6	15
21	0.192	63	4.0	7	20
16	0 157	150	5.5	5	0
Herd	0.153	67	5.0	7	30
19BH	0.153	200	20.0	2	40
22	0.126	115	8.0	4	0
23	0.121	55	4.1	4	50
18	0.112	107	6.0	4	0

Kürsteiner (26) found that reductase activity was greater in milk with a large proportion of cellular elements, and also that Van Slyke and Baker (27) report a greater content of these cellular elements in low acid milk.

There is seen, however, a rather close relation between the speed of rennet reaction and the amount of alcohol necessary for precipitation. These phenomena do not, apparently, depend upon acidity but more likely upon the relationship of the various constituents of milk, similar conditions being favorable to both reactions.

One sample particularly is extreme in this respect—19 BH—requiring an unusual time for coagulation by rennet and also a

large amount of alcohol for precipitation. An analysis made on a sample from the same source but taken at a different time showed the casein to run unusually low and the albumin high. This condition might easily account for the difficulty with which the casein is coagulated by rennet and alcohol. The albumin, acting as a protective colloid, would inhibit the flocculation of casein.

It was pointed out earlier in this paper that concentration of hydrogen ion and acidity by titration vary in the same direction quite regularly. It follows, therefore, that rennet activity here did not seem to be influenced by the former. It is probable, however, that when all other conditions are the same, rennet and alcohol coagulation take place more easily when the acidity is increased. Certain other peculiarities in these samples no doubt influence the coagulation reactions more than hydrogen ion concentration.

SUMMARY AND CONCLUSIONS

Determinations of acidity in milk from a large number of cows have shown variations from 0.086 to 0.229 per cent calculated as lactic acid, and in terms of pH from 6.898 to 6.432.

In general, high acid milk contains more of all the nutrients and is particularly high in phosphates; it has greater food value, therefore, than milk of low acidity.

On account of the fact that it contains a larger proportion of buffer substances, phosphates and casein especially, it would be expected to increase less in hydrogen ion concentration with a given addition of acid or alkali than low acid milk. This was found to be the case. From the point of view of commercial handling of milk this fact is of some importance. High acid milks under the same condition of storage reach the coagulating point later than milk of lower acidity. The following observation illustrates that point: Two samples of fresh milk, one with natural acidity of 0.144 per cent and the other 0.175 per cent were allowed to stand side by side at room temperature. At the end of 9½ hours the first sample coagulated on heating, while the latter did not respond to this test until thirteen hours had elapsed.

Under ordinary conditions of handling, the carbon dioxide varied but little in the different samples. The low acid milk contained, perhaps, a little more. It was calculated that enough free carbon dioxide is usually present to account for the acidity equivalent to 0.01 to 0.02 per cent lactic acid.

Between citric acid and acidity there was found no regular variation. But from experiment it was calculated that acid citrates might account for 0.01 per cent acidity in all samples.

High acid milk contained somewhat more casein yet there was not enough difference to account for more than a small part of the difference in acidity between the high and low acid samples. It was concluded that the acidity due to casein is 0.05 to 0.08 per cent.

The albumin accounts regularly for a little less than 0.01 per cent.

The difference between the total acidity and that due to the sum of the factors just enumerated is attributed to phosphates. Practically the entire difference in acidity between the various samples was shown to be due to this constituent.

The hypothesis is advanced that a condition of equilibrium exists between certain of the milk constituents, particularly citric acid, phosphoric acid, casein, and the bases, and that the acidity due to any one of these depends upon its relation to the others. It is this equilibrium which fixes the hydrogen ion concentration; and a change in the proportion of a constituent or ion will result in a shift in the equilibrium and a change in the influence which each has upon the acidity. The calculations just made, therefore, should not be considered arbitrarily. They represent merely the result of the necessity for using alkali to bring the hydrogen ion concentration of each constituent from that of milk to that point at which the phenol phthalein end-point happens to be.

The decrease in acidity of milk on dilution is due to the hydrolysis of both phosphates and caseinates.

The osmotic pressure of milks of varying degrees of acidity was found to be the same. In low acid milks where lactose is low, the chlorides particularly were high. Thus there is a reciprocity which accounts for the constancy of osmotic pressure.

A fairly close relation between chlorine and conductivity was found as might be expected, though, of course, other salt constituents may conduct the current as well. There is, consequently, an approximate relation between acidity and conductivity, milk with low acidity running high in conductivity.

There was no relation between natural acidity (or pH) and coagulability with rennet and with alcohol. These properties were found, however, to run hand in hand, both probably depending upon certain relations between the constituents in milk which are independent of acidity.

The aldehyde reductase reaction is somewhat more rapid in low acid milks but the relation is only general and is believed to be secondary.

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FACTS ABOUT CARBONATED BUTTER

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Within the last two years numerous inquiries have come to me regarding the merits of carbonated butter. Some of the inquiries pointed out that the promoters of carbonating butter referred to some of my publications as substantiating their sweeping claims of the virtues of carbonated butter. Voluminous advertising propaganda is now conducted in several consuming centers, belaboring the trade and advising the public as to the all-embracing benefits of carbonated butter and the "terrible shortcomings" of butter made in the presence of air. The situation has reached a point where it seems advisable to offer a brief and unbiased statement of the now-known facts of the possibilities and limitations of the process of carbonating butter.

WHAT IS CARBONATED BUTTER?

The so-called carbonating of butter consists of churning the cream and working the butter in an atmosphere of carbon dioxide gas. The churn is loaded with the cream. The gas then is introduced at the bottom of the churn at the end opposite the air vent. The air vent is left open permitting the air to escape while the gas enters. When the gas begins to come out of the air vent, the vent is closed. The cream is then churned and the resulting butter is washed in the usual manner and with the churn open. After the salt has been added the churn is closed, is again filled with the gas, and the working is completed. In the case of the Simplex churn and similar types where the working must be done with the churn open, the butter is worked in the presence of air and the carbon dioxide treatment is confined to the churning operation only.

In short, then, the churn is filled with the carbon dioxide gas. This is all there is to the carbonating of butter, no more, no less. After churning and working, the butter is removed from the

churn and is packed in tubs or boxes in the usual manner and exposed to the air as always. In the earlier stages of the promotion of carbonating butter, it was claimed also that by carbonating his butter the buttermaker could do away with some of the standard and approved processes of manufacture, such as standardization of cream for acidity, pasteurization, the use of starter, etc. A few trial churnings made to determine the dependability of these unusual claims gave such disappointing, though obvious results, that these all-sweeping claims were promptly withdrawn. It should be clear from this, therefore, that the carbonating of butter is a treatment in addition to the generally approved method of butter manufacture. It eliminates or displaces nothing in the normal process of manufacture; it therefore economizes nothing, neither fuel nor labor; if done at all it must be a treatment additional to what we are now doing. It means an added expense of manufacture.

WHAT ARE THE CLAIMS MADE FOR CARBONATED BUTTER?

The following are some of the more outstanding claims made by the promoters of carbonating butter:

1. Conservation of vitamins in butter.
2. Making a mechanically pure butter by keeping dust-laden air out of it.
3. Destruction of bacteria.
4. Improved flavor.
5. Greater keeping quality.

In advancing these claims reference is made by their authors to the name and works of a long list of scientific authorities. Isolated paragraphs from some of these works are quoted and by inference or otherwise it is made to appear that these authorities experimented with butter and that they did find:

That uncarbonated butter loses a large portion of its vitamin strength while carbonated butter conserves it.

That the atmospheric air pollutes the butter with the dust and filth contained in this air, while carbonated butter is free from all these impurities.

That uncarbonated butter is teeming with a great multitude of undesirable bacteria, dangerous to health and disastrous to the quality of the butter, while the carbonating eliminates this danger by destroying the germ life.

That the air in uncarbonated butter inevitably produces rapid oxidation which deteriorates the butter and makes it unfit for consumption, while carbonated butter is free from the danger of such oxidation.

That the carbonated butter has a better flavor and possesses greater keeping quality than uncarbonated butter.

These contentions and inferences, though bulging with monstrous inaccuracies to the point of collapse, sound plausible to the unsuspecting consumer who pays the bill and who wants and asks for butter that is pure and of good quality and, therefore, harmless, safe, and wholesome. Hence these claims furnish advertising thunder and sales talk of a type that proves effective, at least while the camouflage remains intact.

The fact, however, is that these claims are not based on scientific experimentations at all. They are mere theories and hypothetical conclusions. They are the product of an ingenious imagination and do not deserve serious consideration as far as scientific argument is concerned. Their relation to the welfare of the industry, however, compels unbiased and fearless exposure. It is for this reason that each of the above claims will be discussed by itself in the following brief paragraphs:

DOES CARBONATING CONSERVE VITAMINS IN BUTTER?

The contention of the promoters of carbonating is that butter made without carbon dioxide treatment is low in vitamin properties. The argument is that the free oxygen contained in the air with which the cream and butter come in contact during churning and working causes oxidation which in turn destroys a portion of the vitamin properties of the butter. On the other hand, when churning and working in carbon dioxide gas this air is not present, hence oxidation does not take place and the vitamins retain their full strength.

There is no experiment on record, neither in this country nor abroad, to show that the relative vitamin strength of carbonated

and uncarbonated butter has ever been subjected to study. The above claim is purely hypothetical, it is an assumption with no facts to support it whatsoever, but there is an abundance of experimental evidence available showing that the maker of this assumption was ill-advised and that the hypothesis is entirely erroneous.

According to the present status of the newer knowledge of nutrition, the vitamin content of butter is confined very largely to vitamin A (fat soluble A). Other vitamins, if present at all, are there in such small amounts as to be of no consequence. The nutrition experts who discovered vitamin A and experimentally studied its effect on different animals, used butter from which they derived the milk fat that furnished the vitamin A. That butter was made in the ordinary way; that is, in the presence of atmospheric air and not in an atmosphere of carbon dioxide. The great discoveries they made and the wonderful results they obtained in their feeding experiments suggest that vitamin A had lost none of its vitality in spite of the presence of air.

Later they were interested to find out to what extent the deterioration of butter and butter oil would have to progress in order to diminish the activity of vitamin A. Butter was melted and the oil boiled in the air with live steam for several hours without injury, alkalies were added to the oil in the presence of air, enough to make a cake of soap out of it, and still the vitamin remained intact. It was only when butter was exposed to air and light and heat to such an extent and for so long a time that it became bleached and tallowy and utterly unfit for consumption by man or beast that the vitamin strength suffered a decline.

The dominating and conclusive fact here is that the eminent nutrition experts who discovered and experimented with the vitamins contained in butter state unreservedly and unconditionally that the only cases where the milk fat derived from butter had depreciated in its vitamin content were those where it had been the express purpose of the experiment to determine how great an extent of deterioration of butter was necessary before the butter showed a decline in its vitamin strength. And they further state that their feeding experiments demonstrated that there was no

decrease in the vitamin properties of butter resulting from the process of manufacture nor from ordinary commercial cold storage.

These facts are fully borne out also by experiments with ice cream, conducted by the National Association of Ice Cream Manufacturers, under the direct supervision of Dr. Arthur H. Smith, and published in a "Report of an investigation into the effect of freezing ice cream in an atmosphere of carbon dioxide." Dr. Smith summarizes his feeding experiments as follows:

> The experimental findings discussed above represent the results of studies on rats and guinea-pigs extending from May 16, 1922, to September 21, 1922. They indicate that the vitamin A in 1 gram of pasteurized ice cream mixture is the minimal amount which will promote normal growth in white rats fed on an otherwise adequate diet, whether the mixture is fed as such, frozen in air or in an atmosphere of carbon dioxide.
>
> When the ice cream mixture furnishes the sole source of vitamin B for rats on an otherwise adequate diet, 7.7 grams equivalent to 15 grams of milk was required for normal growth. Freezing the mixture in air or in an atmosphere of CO_2 had no effect on the water-soluble vitamin in the mixture.
>
> Guinea-pigs on a scurvy-producing diet were not protected against the disease by the maximal amount of ice cream they could be forced to eat, whether it was frozen in the air or in an atmosphere of CO_2.
>
> Conclusions: From the data at hand we are forced to conclude that there is no evidence supporting the contention that freezing ice cream in an atmosphere of carbon dioxide preserves the vitamins which are present in the unfrozen pasteurized mixture to a greater extent than freezing the ice cream in air.

And so in the case of butter, the vitamins are neither made nor accelerated, nor injured, nor destroyed by any treatment to which the cream or butter may be subjected in the usual operation of the churn, whether this treatment be in air on in an atmosphere of carbon dioxide. Nor does churning and working in air cause any decline in the vitamin strength in butter in commercial cold storage, or under other storage conditions to which butter may be expected to be exposed during the normal passage from creamery to consumer.

This, then, explodes the vitamin bubble of carbonated butter, a beautiful bubble except for the intended and vicious deception of the consuming public regarding the vitamin-superiority of carbonated butter. It does not exist.

IS CARBONATED BUTTER MECHANICALLY PURER THAN NON-CARBONATED BUTTER?

The contention of the promoters of carbonated butter is that the air is very impure, that it is laden with dust and similar impurities. Therefore, so they say, butter made from cream churned in the presence of air is very impure. Carbon dioxide, on the other hand, they claim to be immaculately pure, hence butter churned and worked in an atmosphere of this gas is exceptionally free from impurities.

Here again imagination plays a dominant part in the development of this accommodating hypothesis. There are no data on record that show that butter churned and worked in air contains a larger percentage of these mechanical impurities than butter churned and worked in an atmosphere of carbon dioxide.

Little tangible information is available regarding the relative purity of creamery air and carbon dioxide. However, it is safe to say that the air in the churn at the time the churn is loaded with the cream is relatively pure, the fact is it is washed air. In every well-regulated creamery the churn is steamed out or rinsed out with hot water, and then rinsed with cold water so as to close the pores of the wood and to prevent the butter from sticking to it. The buttermaker would not do without this rinsing because of the mechanical difficulties of the butter sticking to the wood and the obvious injury to the body of the butter, hence rinsing out the churn is as regular a performance as the churning itself. This rinsing is performed by revolving the churn for a few minutes with the water in it. In this operation the air is thoroughly mixed with the water and it would seem reasonable to conclude that the amount of mechanical impurities left in it would be negligible.

Again, after a few minutes of churning the vent is opened in order to release the pressure generated by the liberation of the gases in the cream. In the case of carbon dioxide instead of generating pressure in the churn a partial vacuum is formed due to the action of the CO_2 and when the churn vent or door is opened to release this vacuum the outside air rushes in with great force, carrying into the churn all that dust that is claimed to be in the air.

Also it does seem a little late in the process to try protecting the cream and butter from the mechanical impurities in the air, after the milk is drawn into an open pail in the form of fine streams from the teats and with enough force to whip an inch or more of foam up in the pail. In the centrifugal separator again, air is added and from there on the cream is exposed to air continuously while on the farm, in transit, and in the creamery until it reaches the "saving" atmosphere of carbon dioxide in the churn. When the butter is removed from the churn it is exposed to the air again during packing, printing, wrapping, and more or less until it is consumed.

The National Association of Ice Cream Manufacturers included in its extensive investigation hereinbefore quoted, a study of the dust content of normal and carbonated ice cream. Dr. C. E. A. Winslow, under whose immediate direction this particular phase of the investigation was performed, reports as follows:

> The dust content of the ice cream is not appreciably affected by the Heath System (meaning the carbonating process); no influence of this kind could reasonably be expected since the ice cream contains several thousand dust particles per gram while the air which is mixed with it in the proportion of about one cubic centimeter of air per gram of ice cream contains only between one and two dust particles per cubic centimeter. Comparison of the air in the freezers before the mix was admitted shows that as a matter of fact the air to be mixed with the ice cream in the Heath Process (meaning the carbonating process) actually contained rather more dust particles than were present in the air of the freezer when operated under ordinary conditions.

The above facts and findings are ample to demonstrate the perfect absurdity and pure nonsense of this so loudly-heralded superior purity of carbonated butter.

DOES CARBONATING OF BUTTER DESTROY UNDESIRABLE BACTERIA?

The claim here is that carbon dioxide gas is destructive to bacteria, that carbonating greatly reduces the numerical count and that by so doing the butter is rendered safe from the standpoint of its possible infection with germs of human diseases and that the destruction of other bacteria makes for butter of better keeping quality.

It should be understood here that with reference to the presence or absence of air there are two separate groups of bacteria. To one group belong all those species of microorganisms that must have air in order to live and thrive. To the second group belong the species of germ life that either cannot live or do not thrive in the presence of air, but thrive luxuriantly in the absence of air or in the presence of an inert gas. It is a fact, scientifically well known, that many of the most objectionable and harmful bacteria, such as bacteria that cause putrefaction and the formation of ptomains, or decompose cream with the production of butyric acid, require the absence of air.

That the carbonating of butter does not hinder bacterial deterioration and spoiling of butter was indicated convincingly in split experimental churnings made on a commercial scale in which one-half of the cream was pasteurized and churned in the normal way and the other half was not pasteurized but was given carbon dioxide treatment in the churn. After twelve weeks storage the carbonated butter had become strongly rancid and the score had dropped from 89 points when fresh to 83 points at the end of the twelve weeks, while the uncarbonated butter from the check churning, the cream of which was pasteurized, retained its score of 89 points.

None of our experiments included a study of the effect of carbonating on germs of human diseases. If such germs accidentally were present in the original cream they would not survive the pasteurizing process, which is effectively controlled by a complete system of automatic temperature controllers and recorders.

However, Prucha, Brannon, and Ambrose, who conducted experiments at the University of Illinois under the direction of

Dr. H. A. Ruehe, reported that cultures of Bacillus typhosus inoculated into milk that was subsequently subjected to from 10 to 30 pounds of pressure of carbon dioxide were not destroyed. On the contrary, in four days under 20 pounds of pressure of carbon dioxide gas these bacilli increased from 47,000,000 to 153,000,000 per cubic centimeter of milk.

These investigators further report that

> In comparing the bacterial counts in the plain and in the carbonated ice cream, we are forced to the conclusion that the carbon dioxide gas did not cause any appreciable reduction in the number of bacteria in the carbonated ice cream. If it did destroy any bacteria the number was so small that our method did not detect it. One of the lots of ice cream was kept in the hardening room for six months. Even after this long period of time the carbonated ice cream had just as many bacteria as the plain ice cream.

Prof. Leo F. Rettger of Yale University, who studied "The influence of carbon dioxide on bacteria" for the National Association of Ice Cream Manufacturers, states, "The results obtained in the present investigation are in perfect harmony with the observations of Prucha and his associates in the University of Illinois."

So here again, as in the case of the previously discussed contentions, the claimed miraculous benefit of carbonating disappears in the face of actual scientific facts. Carbonating cannot be depended upon as a means of destroying harmful or injurious bacteria that may be present in the cream and of rendering such cream or the butter made from it safe for human consumption. Nor can carbonating be relied upon to destroy bacteria that are harmful to the flavor and keeping quality of the butter. Carbonating is incapable of taking the place of pasteurization. Without pasteurization carbonated butter becomes rancid and develops other objectionable bacterial flavor defects quickly and intensely, as is the case with uncarbonated butter made from unpasteurized cream. Carbonating does not improve the bacterial status of butter.

DOES CARBONATING IMPROVE THE FLAVOR OF BUTTER?

The contention here is that carbonated butter, being "purer" than ordinary butter, has a cleaner flavor, and because of the flavor-accentuating tendency of carbon dioxide, has a more pronounced pleasing flavor.

In our experiments in which churnings from the same vat were used for both treated and untreated butter, and in which the judges had no knowledge of the treatment that had been given the cream and the butter in the churn, the scores showed no difference between carbonated and ordinary butter. In split churnings where the untreated butter scored 91 points, the carbonated butter also scored 91 points, except in the case of butter where the carbonating was done in the place of our regular method of neutralization and pasteurization instead of in addition to our regular method, and in this case the carbonated butter scored one point lower than the untreated butter.

The above represents results of experiments carried out with exactness. The purpose was to secure unbiased facts. A vast number of other creameries could be cited to whom the attractive and convincing contentions of the promoters of the carbonating process appealed sufficiently to give the process a try-out, but who soon were forced to realize that the much advertised superiority of carbonated butter did not materialize; they abandoned the manufacture of carbonated butter because the product failed to make good.

And so once again the conclusion is compelling that a vital claim for carbonated butter falls by the way side. Carbonating does not improve the flavor of butter in the sense of increasing its market value. It is possible that the butter at the churn may possess a slightly accentuated snappiness of flavor that pleases the palate. If this is the case, the favorable effect is of too short duration to be of commercial value, as clearly indicated above by the agreement in score between carbonated and uncarbonated butter.

DOES CARBONATING IMPROVE THE KEEPING QUALITY OF BUTTER?

The contention of the promoters of carbonating here is that because carbonating "reduces the bacterial count" and because it "prevents" oxidation, the factors that are responsible for butter deterioration are largely eliminated, hence carbonated butter keeps better.

It has already been shown under the caption "Does Carbonating of butter destroy undesirable bacteria?" that the absence of air brings about a condition favorable for some of the most objectionable groups of bacteria such as those whose activity means putrefaction and other harmful decomposition.

As previously stated, our own experiments demonstrated that carbonated butter that is not made from properly pasteurized cream has no keeping quality at all. The carbonated butter became rancid and dropped from 6 to 7½ points in twelve weeks, while the untreated butter made in the regular way and kept under the same conditions showed the usual good keeping quality. This merely emphasizes the fact that carbonating cannot be relied upon to make butter keep as far as prevention of bacterial deterioration is concerned.

Another important agency that influences the keeping quality of butter is oxidation. It is well known and fully recognized by dairy scientists that oxidation is one of the outstanding causes of butter deterioration. Again wholly disregarding scientific facts, the promoters of carbonating proclaimed that churning in an atmosphere of carbon dioxide would eliminate deteriorating oxidation in butter.

If the cream were pasteurized, churned, and the butter washed, worked, packed, and hermetically sealed in an atmosphere of carbon dioxide, there might be some real merit to carbonating butter from the standpoint of eliminating the oxidizing action of the air. But as it is, the carbonated butter is laboriously manipulated in air after it leaves the churn and no attempt whatsoever is made to prevent the carbon dioxide from escaping and the air from entering in its place. The butter is taken out of the churn, is packed into tubs or cubes, tamping vigorously each handful

as it is thrown into these receptacles, and later on much of this butter is cut up into prints with still more surface exposed to the air. Obviously, much of the carbon dioxide is thus pounded out and into its place much air is pounded into the butter.

It stands to reason, therefore, that the supposed protection by carbon dioxide is very largely forfeited and the so-called carbonated butter has sufficient contact with air to make possible oxidation through air.

However, air is only one of the many factors that under certain conditions may assist in deteriorating butter through oxidation. The newer knowledge of butter deterioration recognizes that in the problem of preventing chemical deterioration in commercial butter the air is sufficiently eliminated in the regular method of manufacture and packing to constitute a negligible factor as compared with other and more important factors and combinations of factors, such as acidity of cream, and the presence of metals and metallic salts and oxides. If the process of manufacture is so regulated that the acidity is standardized to the proper point, and the cream and butter are protected against excessive exposure to metals that are active oxygen carriers and catalyzers, there is very little danger from oxidation and other chemical action that leads to the deterioration of the butter with age. If these factors are not taken care of, then mere churning of the butter in an atmosphere of carbon dioxide is of no avail and means nothing more than wasting gas and time.

Actual experiments in which the keeping quality of carbonated butter was compared with that of uncarbonated butter are very limited. What data we have show that if butter is made right as to acidity, pasteurization, and protection against the action of metals, it will have equally good keeping quality whether carbonated or not. If the above factors are neglected or ignored altogether the butter will not keep, it will deteriorate rapidly, whether carbonated or not.

SUMMARY

1. The vitamins in ordinary butter are not diminished by the regular processes of manufacture, nor are they weakened as the result of commercial cold storage.

The contention that carbonated butter is richer in vitamins than butter made in the regular standard way has no foundation whatsoever. It is a myth.

2. There is no evidence to show that butter churned and worked in air contains more mechanical impurities than butter churned and worked in an atmosphere of carbon dioxide.

The available facts indicate that the contention that the mechanical purity of carbonated butter is superior to that of uncarbonated butter is purely hypothetical and completely devoid of scientific facts.

3. Carbonating cannot be relied upon as a means of destroying harmful and injurious bacteria that may be present in the cream and rendering such cream or the butter made from it safe for human consumption.

Carbonating cannot be relied upon to destroy bacteria that may be present in the cream and that are harmful to the flavor and keeping quality of the resulting butter.

Carbonated butter develops the usual bacterial flavor defects if made from unpasteurized cream.

4. Carbonating does not improve the flavor and market value of butter. It may accentuate the flavor slightly but the effect is of short duration only and not sufficiently recognizable to affect its market value.

5. Carbonated butter keeps no better than butter not carbonated. If the method of manufacture as relating to neutralization, pasteurization, and protection from metals and metallic salts is right, the keeping quality of carbonated and uncarbonated butter is equally good. In the absence of these standard and approved methods carbonated butter will not keep.

6. These facts show conclusively that the pretended superiority of carbonated butter does not exist.

SWEETENED CONDENSED MILK

II. A COMPARATIVE STUDY OF METHODS FOR DETERMINING TOTAL SOLIDS[1]

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Sweetened condensed milk on account of its high sucrose content is generally considered one of the most difficult dairy products to test for total solids. In the past the official procedure of the Association of Official Agricultural Chemists has usually been employed, while more recently the Mojonnier method has come into common use.

So far, however, little study has been made as to the relative accuracy of the Mojonnier method as compared with the official. The only reference found in scientific literature is by Mojonnier and Troy (Technical Control of Dairy Products). One batch of condensed milk was tested by six operators using the Mojonnier method. The results obtained by these men were then checked against the official method as applied by still another operator. While their results seem to indicate close agreement between the official and the Mojonnier, the data is rather too meagre to allow drawing conclusions.

Furthermore, both these methods, while perhaps the best available at present, have certain disadvantages, which limit their use in many instances. The official method, although undoubtedly accurate and reliable, is laborious and time consuming, requiring from six to eight hours for completion. The Mojonnier method on the other hand, while rapid, necessitates

[1] The work reported in this article was done in the Laboratory of Chemistry, Cornell University.

the use of expensive equipment which frequently is beyond the financial means of a laboratory or commercial firm.

In view of these facts it was thought desirable to run a series of trials in order to check the Mojonnier method against the official, as well as to attempt to find a simple and accurate method which might possess advantages that these do not have.

EXPERIMENTAL WORK

Twenty samples of condensed milk were analysed by three methods: (1) the official, (2) the Mojonnier, and (3) a modified method suggested by the authors.

The procedure of each method was as follows:

1. *Official method.* The Association of Official Agricultural Chemists method as described in Methods of Analysis of the Association of Official Agricultural Chemists, page 231, was followed except that 20 grams of condensed milk were made up to 100 cc. with redistilled water instead of 100 grams to 500 cc. as outlined. Clean sand was added to the dishes to facilitate drying.

2. *Mojonnier method.* The procedure for determining total solids in sweetened condensed milk as described in Technical Control of Dairy Products by Mojonnier and Troy, page 125, was followed exactly.

The alternative is given of drying the samples for only twenty minutes and applying correction by deducting 0.3 from the final per cent of total solids or of drying the sample for ninety minutes. In these trials, samples were dried for both twenty minutes and ninety minutes for comparison.

3. *Modified method.* Twenty-five hundredths to 0.35 gram portions of thoroughly mixed sample were weighed into previously dried and tared aluminum dishes. Two cubic centimeters of hot distilled water was added and after mixing, the dishes were placed on an electric hot plate kept at about 180°C. and the solution carefully evaporated until first traces of brown appeared. Drying was then continued in an oven at the temperature of boiling water until constant weight was reached.

The dishes used in this test were ordinary flat-bottomed aluminum dishes about 5 cm. in diameter. Empty dishes were dried for ten minutes in the water oven and then cooled for a similar period in an ordinary calcium chloride desiccator; they were always kept covered while weighing.

The percentages of total solids obtained by each of the three methods are reported in table 1. In interpreting and analysing the data the nature of the product tested should always be considered. Containing from 70 to 74 per cent solids in which 40 to 45 per cent is sucrose, it is practically impossible to obtain results checking as closely as some other products such as whole milk, evaporated milk and ice cream. Bearing this in mind the three

TABLE 1

Percentage of solids in samples of condensed milk according to the three methods studied

SAMPLE NUMBER	OFFICIAL METHOD	MODIFIED METHOD	MOJONNIER	
			At 20 minutes	At 90 minutes
1a	73.09	73.35	73.63	73.64
1b	73.15	73.49	73.74	73 64
2a	72.32	72.96	72.95	73.03
2b	72.40	73.02	72.97	72.97
3a	72.86	72.87	73.20	73.15
3b	72.72	72.95	73.22	73.20
4a	71.05	71.97	71.54	71.44
4b	71.11	71 92	71.50	71.39
5a	72.73	73.67	73.35	73.35
5b	72.72	73.68	73.41	73.43
6a	71.90	72.21	72.95	72.94
6b	71.92	72.13	72.90	72.96
7a	73.47	73.45	74.05	73.93
7b	73.39	73.46	74.06	74.10
8a	72.64	72.80	72.99	72.91
8b	72.56	72.96	73.06	72 99
8c	72.72	73.31	72.93	72.99
8d	72.43	73.19	72.91	72.80
9a	71.82	72.33	72.56	72.72
9b	71.73	72.43	72.49	72.49

tests show reasonably close correlation in results. In all instances except one, the official method gave lower results than the Mojonnier or the modified. It is further interesting and significant that in more than two-thirds of the cases the Mojonnier method was the highest of all, while the modified test gave results usually between the official and the Mojonnier.

A better analysis can perhaps be made from table 2 in which are tabulated the differences obtained in the three methods. Com-

pared with the official method the Mojonnier invariably gave higher results. On the 20 samples the average was 0.57 per cent higher. In the case of the modified method the average was 0.47 per cent above the official.

TABLE 2

Variation in percentage of solids in condensed milk as obtained by the three methods studied

SAMPLE NUMBER	MODIFIED FROM OFFICIAL	MOJONNIER FROM OFFICIAL	
		At 20 minutes	At 90 minutes
1a	+0.26	+0.54	+0.55
1b	+0.34	+0.59	+0.49
2a	+0.64	+0.63	+0.71
2b	+0.62	+0.57	+0.57
3a	+0.01	+0 34	+0 29
3b	+0 23	+0.50	+0.48
4a	+0.92	+0.49	+0.39
4b	+0.81	+0.39	+0.28
5a	+0.94	+0 62	+0.62
5b	+0.96	+0.69	+0.71
6a	+0.31	+1.05	+1.04
6b	+0.21	+0.98	+1.04
7a	−0.02	+0.58	+0.46
7b	+0.07	+0.67	+0.71
8a	+0.16	+0.35	+0.27
8b	+0.40	+0.50	+0.43
8c	+0.59	+0.21	+0.27
8d	+0.76	+0.48	+0.37
9a	+0.51	+0.74	+0.90
9b	+0.70	+0.76	+0.76
Average variation	0.47	0.57	0.57
	55 per cent within 0.5 per cent of official — 80 per cent within 0.75 per cent of official	30 per cent within 0.5 per cent of official — 85 per cent within 0.75 per cent of official	30 per cent within 0.5 per cent of official — 80 per cent within 0.75 per cent of official

Taking the official method as the standard for comparison the modified method here proposed gives more favorable results than the Mojonnier. It furthermore is simpler and more rapid

than the official. The equipment needed is inexpensive and frequently on hand in the laboratory.

In order to determine the average time for samples in the modified test to reach constant weight, careful record as to time of drying was kept in the case of 14 samples. The dishes containing the condensed milk solids were first weighed at the end of two hours drying in the oven and then again at intervals of 30 minutes until constant weight was reached.

TABLE 3

Time required for solids in condensed milk to reach constant weight in modified test

SAMPLE NUMBER	PERCENTAGE OF MOISTURE LOST AT VARIOUS PERIODS OF DRYING IN WATER OVEN				
	0 to 2 hours	2 to 2½ hours	2½ to 3 hours	3 to 3½ hours	3½ to 4 hours
1a	26.65	0	0	0	0
1b	26.25	0.10	0.13	0.02	0.01
2a	27.04	0	0	0	0
2b	26.93	0.05	0	0	0
3a	27.03	0.10	0	0	0
3b	27.05	0	0	0	0
4a	27.87	0.12	0.04	0	0
4b	27.69	0.26	0.13	0	0
5a	26.33	0	0	0	0
5b	26.03	0.21	0.08	0	0
6a	26.50	0.86	0.42	0.01	0
8c	26.69	0	0	0	0
8d	26.64	0.17	0	0	0
9a	26.76	0.59	0.29	0.03	0

Results as given in table 3 indicate that while in the majority of cases three hours is sufficient time to reach constant weight, there are occasional samples which require a longer period. Such factors as size of sample and humidity of the air materially affect the rate of drying. On humid days great care must be exercised to weigh and handle dishes quickly in order to prevent moisture being absorbed from the air. All factors considered, it is best, therefore, to dry the samples for three and a half hours, after which one can be reasonably certain that all moisture has been removed.

SUMMARY AND CONCLUSIONS

1. Twenty samples of condensed milk were tested for total solids by the official, the Mojonnier and a modified method suggested by the authors.

2. The Mojonnier method, invariably gave higher results than the official; in 20 samples the average difference was 0.57 per cent.

3. In the Mojonnier method the correction of 0.3 per cent applied at the end of twenty minutes drying in the oven checked closely with the final results at ninety minutes.

4. The modified method also gave somewhat higher results than the official; the average of 20 samples was 0.47 per cent above.

5. In more than two-thirds of the cases, the Mojonnier test was the highest of all, while the modified method usually gave results between the official and the Mojonnier.

6. The time required for 14 samples to reach constant weight in the modified method was between three and three and a half hours, while in the official method it required from five to six hours.

7. The modified method recommends itself as simple and economical for the determination of total solids, and as reliable as the Mojonnier.

THE DISPOSAL OF DAIRY WASTES[1]

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The dairy industry is one which is very widely distributed, because the nature of the raw material limits the time which may elapse between production and consumption. To make this time as short as possible, long-distance transportation of raw milk is not usually practised and therefore local means must be provided for its reception and rapid distribution. Accordingly, scattered over the country are many small dairy plants as well as numerous ones of considerable size. These may be merely receiving or skimming stations, where the character of the milk is not greatly changed; or, on the other hand, they may be creameries, condenseries, cheese factories, butter factories, milk-sugar factories or casein plants, where the raw milk is converted into other products.

When these plants are situated near a city with sanitary sewers, or when large water courses are available to receive it, no difficulty has arisen regarding the disposal of milk waste. But where no sewers are available and a ditch or small stream must receive the waste, nuisances have arisen which, in dry summer weather particularly, have been extremely objectionable.

Several such nuisances having arisen in Maryland, Mr. Abel Wolman, Chief of the Bureau of Sanitary Engineering of the State Department of Health, presented the problem to the Bureau of Chemistry and requested its solution. He suggested that it might be solved by accurately controlling the pH value of the waste. Acknowledgment is hereby made of his suggestions and criticisms. The ultimate solution of the problem was made

[1] A dissertation presented by the author in partial fulfilment of the requirements for the degree of Doctor of Science in Hygiene in the School of Hygiene and Public Health of the Johns Hopkins University.

possible by Dr. W. W. Randall, Chief of the Bureau of Chemistry of the same Department, who so arranged the routine work of the laboratory that the necessary time could be given to this research. Grateful acknowledgment is made of his interest and encouragement at all times.

The disposal of creamery wastes has been the subject of many investigations both in this country and abroad. These have generally been concerned with the wastes from large plants where certain devices, commonly used in sewage purification, have been utilized. Until 1920 this method had not proven satisfactory, but with specially constructed tanks, filters, etc.,—such as have been recommended by Hommon in Public Health Bulletin No. 109(1),—the results obtained were satisfactory enough to demonstrate that the method could be applied, perhaps with modifications, to other types of dairy wastes than had been studied in that investigation.

While it is possible that this method is proving satisfactory for the large plants, it is too expensive and involved to be practicable for small plants or receiving stations. Yet the waste from these and also from skimming stations, even though comparatively small in amount, has given rise to considerable complaint.

This study was undertaken in the hope of finding a simple and easily applied method of abating this nuisance.

The total solids in milk average about 12.9 per cent: fat, 4 per cent; proteins, 2.8 per cent; lactose, 4.75 per cent (3). Lactose and milk fat are fairly stable substances, but proteins as a class are unstable and very readily undergo decomposition. On the assumption that the nuisance was caused by the decomposition of the proteid material, the problem became one of the separation and disposal of protein, with a subsequent study of the relative stability values of the raw and treated waste.

Casein constitutes 80 per cent of the total proteins in milk, therefore conditions were sought which would best serve to remove the casein. No particular attention was given to the other proteins, although it was realized that the fat would also be carried down by the casein during sedimentation, leaving only the lactose and minute amounts of albumin and globulin in the solution.

A review of the literature on proteins and, more particularly, on casein, gave the following reagents as available precipitants:—a saturated solution of sodium chloride, of ammonium sulphate, or of magnesium sulphate, at ordinary temperature; small amounts of alum, of zinc sulphate, or of other metallic salts; calcium chloride and other salts at 34° to 45°; rennet; acids, particularly acetic (2); ferric sulphate (134 grains per gallon); lime to neutrality, with sodium silicate then added (1).

To meet the practical demands of this problem it was required that the precipitating agent be easily available, comparatively inexpensive, and its employment simple in operation. For these reasons the following precipitants were considered: calcium chloride, alum, alum and sulphuric acid, and sulphuric acid alone, with and without the application of heat. It was desired to dispense with filtration, if possible, so that sedimentation or precipitation became of as great importance as flocculation.

After consultation with a dairyman familiar with both large and small plants, it was estimated that the wash-water from a receiving station would contain approximately 0.25 to 0.5 per cent milk. Since even this small amount had caused considerable nuisance, the first experiments were made with these concentrations. In order that the experiments might be as uniform as possible in the beginning, distilled water was used for making the dilutions. Raw milk was supplied in part by the Western Maryland Dairy of Baltimore, and partly by the ordinary laboratory samples which had been received for other analyses. Later, certified milk was purchased from the Walker Gordon Laboratory and used for all determinations. It was thought necessary to use raw milk rather than pasteurized in the experiments for two reasons: first, the difference in bacterial flora would probably cause a difference in the relative stability numbers; and second, owing to pasteurization, the proteins might possibly be in various states of aggregation not found in raw milk, from which the nuisance arose.

A small number of preliminary experiments were set up as follows:

		p.p.m.
(1)	500 cc. $\frac{1}{2}$ per cent milk solution plus calcium chloride...........	5
(2)	500 cc. $\frac{1}{2}$ per cent milk solution plus calcium chloride...........	10
(3)	500 cc. $\frac{1}{2}$ per cent milk solution plus calcium chloride...........	15
(4)	500 cc. $\frac{1}{2}$ per cent milk solution plus calcium chloride...........	20

These were kept at room temperature (approximately 20°) over night, when no. 3 and no. 4 showed slight flocculation but no sedimentation. Other samples similar to no. 3 and no. 4 were heated to 30°, 40°, and 50°, as well as lowered in temperature to 12°, but a satisfactory floc was not obtained. Further work with calcium chloride was therefore not undertaken.

Casein is an amphoteric electrolyte whose isoelectric point is pH 4.6(4), while the optimum floc formation of alum is at pH 5.5(5). Now in the filtration of domestic sewage, of isoelectric point pH 3.2, it has been found that, when alum is added, the time of filtration is greatly shortened if the isoelectric point is shifted to approximately pH 4.4, the mean of the two isoelectric points(6). Therefore with alum of isoelectric point pH 5.5 and casein of pH 4.6 it might be expected that the best flocculation would occur at pH 5.0 or 5.1.

Accordingly, the following experiments were tried: (I) 500 cc. of $\frac{1}{2}$ per cent milk solution plus alum until pH 5.1 was reached. This required 460 drops of alum, approximately 230 p.p.m. The floc formed almost immediately and settled overnight leaving a clear supernatant liquid. (II) 500 cc. of $\frac{1}{2}$ per cent milk solution plus acid to pH 5.1, plus 10 drops of alum, approximately 5 p.p.m. The floc formed in a few minutes, smaller than in I, but leaving a clear supernatant liquid after standing overnight. Accordingly a more detailed study was made of alum with and without the addition of acid:

0.5 per cent milk solution	Alum	pH	Floc	Acid	pH	B floc	Sedimentation overnight
cc.	cc.			cc.			
500	0 5	6.8	—	1	5.2	No floc	None
600	1.0	6.8	—	1	5.2	No floc	None
500	1.5	6.8	—	1	5.1	10 minutes	Good
500	2.0	6.6	—	1	4.8	10 minutes	Good
500	2.5	6.6	—	1	4.6	35 minutes	Good
500	3.0	6.6	—	1	4.4	Overnight	Poor
500	3.5	6.5	—	1	4.4	No floc	None

When an hour had elapsed after the addition of alum and no floc had appeared 1 cc. N/10 acid was added to each sample with the results shown in portion B of the table.

Then the following samples were set up:

0.5 PER CENT MILK SOLUTION	C				SEDIMENTATION OVERNIGHT
	Acid	Alum	pH	Floc	
cc.	*cc.*	*cc.*			
500	1	1.0	5.1	Overnight	Poor
500	1	1 5	4.8	10 minutes	Good
500	1	2.0	4.7	10 minutes	Good
500	1	2 5	4.6	2 minutes	Good

At the very beginning of this investigation it had been anticipated that the proper adjustment of the pH value of the milk waste would be the best means of coagulating and precipitating the casein. This seems to be borne out by the above tabulated experiments, which indicate that the alum of itself does not cause or aid flocculation except as it effects the pH value of the solution. Therefore further study with alum was not continued, acid alone being used as the precipitant.

The following samples were then set up:

0.5 PER CENT MILK SOLUTION	ACID	pH	FLOC	SEDIMENTATION OVERNIGHT
cc.	*cc.*			
500	1.3	5.4	No floc	None
500	1.4	5.3	No floc	None
500	1.5	5.1	11 minutes	Good
500	1.6	4.9	1½ minutes	Good
500	1.7	4.7	2 minutes	Good
500	1.8	4.6	8 minutes	Good
500	2 0	4.4	Overnight	Poor

These results indicate that between pH 5.1 and 4.6 the casein will be precipitated.

All the foregoing pH values may be slightly incorrect owing to a slight acidity in the methyl-red indicator and an incomplete set of buffer solutions for comparison. But as these experiments were merely preliminary ones, it was thought best to include the results, particularly since they indicate the reason acid alone was more extensively studied as the precipitant.

At this point a set of buffer solutions pH 4.2 to pH 6.2 were made, using the phthalate-sodium hydroxide mixtures as given by Clark(7). All the precautions known were taken in the preparation of these, and as the author had formerly made such mixtures which checked with potentiometer measurements, it is believed that these solutions are within the limits of accuracy for readings made with color standards. The alcoholic solution of methyl-red used as indicator was carefully neutralized, and all test tubes cleansed with acid, tap water and distilled water before any readings were made. In each experiment the time of first appearance of floc was noted, as it has been found that "the floc which appears in the shortest time, in any series, is also the one which possesses the qualities of rapid settling and abundance in the highest degree" (5). Accordingly, each floc was numbered, beginning with 1 for that which appeared in the shortest time, and continuing upward for as many different samples in any series as showed any flocculation. This method was adopted rather than the absolute time of flocculation, for, as more experience was obtained in the observation of floc formation, the time became shorter. In the beginning flocculation was observed by tilting the beaker containing the solution so that only a small layer of liquid at the side was observed. Since the observations were made on a table having a black top the floc was easily discerned. When larger volumes were used this method proved impracticable, so an ordinary glass tube, of 4 to 6 mm. bore, was used in which a small portion of the liquid was withdrawn by closing one end of the tube with the forefinger. By carefully wiping off the outside of the tube and looking through a cross-section it was found a simple matter to determine whether or not floc had formed.

The method of procedure was now as follows: 500 cc. of the dilute milk solution, either 0.5 or 0.25 per cent, was poured into an 800-cc. beaker; acid was added from a burette, the time recorded and the solution stirred vigorously. Ten cubic centimeters were at once transferred to a test tube containing a measured quantity of methyl-red indicator. The color produced was then compared with the colors produced by buffer solutions of known pH values plus methyl-red. The color comparisons were made in a simple

comparator, similar to that described by Clark(7) as the "comparator of Hurwitz, Meyer and Ostenberg" (1915). However a paste-board box made by Dr. Randall, was used instead of a block of wood: its construction was simple and in practise it proved satisfactory.[2]

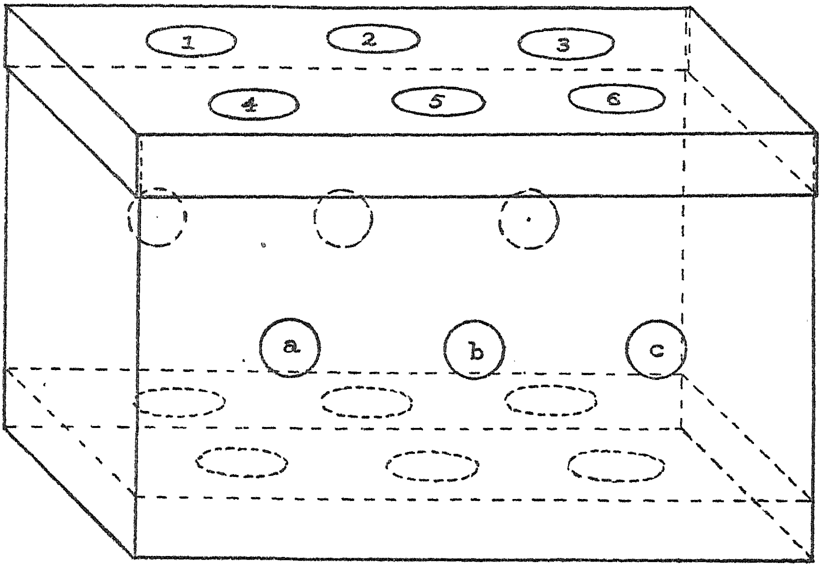

FIG. 1

The following experiments pp. 510–512 were made using 500 cc. of $\frac{1}{2}$ per cent milk solution and $\frac{N}{10}$ sulphuric acid.

[2] The exact size of the box is unimportant, but 6 inches long, 3 inches wide and $3\frac{1}{2}$ inches deep, are good dimensions. In the top of the box are cut six holes, in three pairs, each hole being just large enough to accommodate a test tube. The box is provided with a double bottom, in the upper one of which are cut six holes corresponding to those in the top. In the front and in the back of the box are cut three holes, respectively opposite, through which the light enters and the tubes are viewed. The entire interior is coated with black so that when test tubes are in each hole the only light admitted is through the front and back holes. In holes 1 and 3 tubes of milk solution are placed, in holes 4 and 6 the color standards, in hole 2 a tube of distilled water and in hole 5 a tube of milk solution, plus indicator, whose pH value is desired. The tubes are then viewed through the holes a, b, and c and the standards changed until one of them matches or nearly matches the unknown.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		May 10		
cc.		*minutes*		
1.4	5.1	No floc		None
1.5	4.9	13.5	4	Good
1.6	4.8	9.0	1	Good
1.7	4.7	12.5	3	Good
1.8	4.6	9.0	1	Good
		Repeated May 10		
1.5	5.0	8.0	4	Good
1.6	4.8	4.0	2	Good
1.7	4.65	3.5	1	Good
1.8	4.55	4.5	3	Good
		May 11		
1.5	4.9	2.5	2	Good
1.6	4.8	2.0	1	Good
1.7	4.6	2.5	2	Good
1.8	4.55	3.5	4	Good
		May 18		
1.5	5.0	3.0	3	Good
1.6	4.9	1.5	1	Good
1.7	4.8	1.5	1	Good
1.8	4.6	4.0	4	Good
		May 23		
1.4	5.2	21 0	5	Good
1 5	5.1	10.0	4	Good
1.6	5.0	9.0	3	Good
1.7	4.9	7.0	1	Good
1.8	4.8	8.0	2	Good
1.9	4.7	29.0	6	Good
		May 31		
1.3	5.3	No floc		None
1.4	5.2	No floc		None
1.5	5.1	21.0	5	Good
1.6	5.0	1.75	3	Good
1.7	4.9	1.15	2	Good
1.8	4.9	1.10	1	Good
1.9	4.8	8.10	4	Good
2.0	4.7	38.0	6	Good

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		June 6		
cc.		*minutes*		
1.4	5.2	18.0	6	Good
1.5	5.1	7.5	4	Good
1.6	5.0	3.0	2	Good
1.7	4.9	2.0	1	Good
1.8	4.8	4.0	3	Good
1.9	4.6	9.0	5	Good
2.0	4.5	Overnight	7	Poor
2.1	4.4	No floc		None
		June 11		
1.3	5.2+	No floc		None
1.4	5.2	No floc		None
1.5	5.1	18.0	5	Good
1.6	5.0	9.0	3	Good
1.7	4.9	7.0	1	Good
1.8	4.8	10.0	4	Good
1.9	4.6	7.0	1	Good
		February 4		
1.9*	5.2	No floc		None
2.0	5.0	38.45	5	Good
2.1	4.8	5.20	1	Good
2.2	4.7	6.30	2	Good
2.3	4.6	7.10	3	Good
2.4	4.5	7.45	4	Good
2.5	4.4	No floc		None
		February 7†		
1.8	5.15	180.0	6	Poor
1.9	5.05	3.5	4	Good
2.0	4.8	1.0	1	Good
2.1	4.7	2.5	2	Good
2.2	4.7	3.0	3	Good
2.3	4.6	7.0	5	Good
2.4	4.5	No floc		None
2.5	4.4	No floc		None

* In this and the two following tables 1.25 per cent acid was used.

† In these experiments good sedimentation was obtained, except in the first and last two instances, after five hours, indicating that a detention period of five to six hours might be sufficient.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		February 11		
cc.		*minutes*		
1.8	5.3	No floc		None
1.9	5.1	6.0	2	Good
2.0	4.9	3.0	1	Good
2.1	4.6	35.0	3	Good
2.2	4.4	42.0	4	Good
2.3	4.4	Overnight	5	Fair

Summary of flocculation scores for ½ per cent solution using volume of 500 cc.

pH	4.5	4.55	4.6	4.65	4.7	4.8	4.9	5 0	5.1
Average score	4.0	3.5	2.9	2.0	2.0	2.1	1.8	3.3	4.0

The same experiments were tried with 500 cc. of 0.25 per cent milk solution.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		May 16		
cc.		*minutes*		
0.7	5.2	Overnight	4	Poor
0.8	5.1	78	1	Good
0.9	4.8	78	1	Good
1.0	4.6	198	3	Fair
1.1	4.5	No floc		None
		May 18		
0.7	5.4	No floc		Fair
0.8	5.2	53	3	Good
0.9	5.0	28	1	Good
1.0	4.8	28	1	Good
1.1	4.6	Overnight	4	Good
		May 23		
0.8	5.2	No floc		None
0.9	4.8	29	1	Good
1.0	4.7	40	2	Poor
1.1	4.6	No floc		None

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		May 31		
0.8	5.1	35	3	Overnight
0.9	5.0	31	1	Not observed
1.0	4.8	34	2	Not observed
1.1	4.7	38	4	Not observed
1.2	4.6	46	5	Not observed
		June 6		
0.8	5.2	41	3	Not observed
0.9	5.0	27	1	Not observed
1.0	4.8	30	2	Not observed
1.1	4.7	Overnight	4	Not observed
1.2	4.6	No floc		Not observed
1.3	4.6	No floc		Not observed
		February 11		
1.6*	5.2	No floc		None
1.7	4.7	71	1	Good
1.8	4.5	198	2	Fair
1.9	4.4	198	3	Fair

* In this table and in all that follow 1.25 per cent acid was used.

Summary of flocculation scores for 0.25 per cent solution, using a volume of 500 cc.

pH	4.4	4.5	4.6	4.7	4.8	5.0	5.1	5.2
Average score	3.0	2.0	4.0	2.7	1.4	1.0	2.0	3.3

In an effort to test the "volume effect" upon floc formation the volume of the dilute solution was increased five times, i.e., to 2500 cc. and experiments carried out as before.

0.5 per cent solution, volume 2500 cc.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		June 18		
cc.		*minutes*		
3.2	5.0	15	3	Good
3.4	5.0	4	1	Good
3.5	4.9	5	2	Good
3.6	4.9	21	4	Good
3.7	4.8	Overnight	5	Good

By this time it was believed that flocculation by means of sulphuric acid was certain, and over a fairly wide range of pH values—5.2 to 4.6. So that it was now thought advisable to make all dilute solutions with tap water, instead of distilled water, more nearly to approach plant conditions, and to determine approximately the amount of acid which a small plant would require.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
January 8				
cc.		*minutes*		
11.0	5.2	No floc		None
11.2	5.1	226.0	10	Good
11.4	5.05	102.0	9	Good
11.6	5.0	52.0	8	Good
11.8	4.9	21.5	7	Good
11.9	4.9	17.0	6	Good
12.0	4.8	10.5	5	Good
12.1	4.7	6.0	3	Good
12.2	4.65	3.0	2	Good
12.3	4.6	2.0	1	Good
12.4	4.5	9.0	4	Good
Repeated January 8				
11.4	5.1	131.0	9	Good
11.6	5.0	31.0	8	Good
11.8	4.9	29.0	7	Good
11.9	4.9	26.0	6	Good
12.0	4.8	24.0	5	Good
12.1	4.8	19.0	4	Good
12.2	4.7	5.0	2	Good
12.3	4.7	4.0	1	Good
12.4	4.65	5.0	2	Good
12.5	4.5	No floc		None
Repeated January 8				
11.6	5.0	5.0	6	Good
11.8	4.9	3.5	4	Good
12.0	4.85	2.0	1	Good
12.1	4.8	4.0	5	Good
12.2	4.75	2.5	2	Good
12.3	4.65	3.0	3	Good

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		January 15		
cc.		*minutes*		
10.0	4.95	15.0	5	Good
10.2	4.9	17.5	6	Good
10.4	4.9	8.5	4	Good
10.6	4.85	2.0	1	Good
10.8	4.8	5.0	2	Good
11.0	4.7	5.5	3	Good
		February 4		
9.4	5.2	No floc		None
9.6	5.2	153.0	9	Poor
9.8	5.1	45.0	8	Good
10.0	4.9	1.53	4	Good
10.2	4.85	3.08	5	Good
10.6	4.8	0.91	2	Good
10.8	4.7	4.7	6	Good
11.0	4.6	0.8	1	Good
11.2	4.4	1.08	3	Good
11 4	4.3	30.0	7	Good
		Repeated February 4		
9.8	5.1	48.0	9	Good
10.0	5.0	25.0	8	Good
10 2	4.9	5.66	5	Good
10.4	4.8	5.55	4	Good
10.6	4.7	1.08	1	Good
10.8	4.6	1.25	2	Good
11.0	4.5	6.25	6	Good
11.2	4.45	9.43	7	Good
11.4	4.4	4.33	3	Good
11.6	4.4	92.0	10	Good

Summary of flocculation scores for 0.5 per cent solution, using a volume of 2500 cc.

pH................	4.4	4.5	4.6	4.65	4.7	4.75	4.8	4.85	4.9
Average score......	3.0	4.0	1.0	2.3	2.0	1.0	3.8	2.3	4.2

0.25 per cent solution, volume 2500 cc.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		February 11		
cc.		*minutes*		
8.0	5.2	103	7	Not observed
8.2	5.0	62	3	Not observed
8.4	4.8	60	2	Not observed
8.6	4.8	55	1	Not observed
8.8	4.7	83	4	Not observed
9.0	4.6	83	5	Not observed
9.2	4.4	87	6	Not observed
9.4	4.3	160	8	Not observed
		February 15		
8.4	5.2	No floc		None
8.6	5.0	41.0	3	Good
8.8	4.8	39.0	2	Good
8.9	4.7	37.0	1	Good
9.0	4.6	42.0	4	Good
9.1	4.4	42.5	5	Good
9.2	4.35	43.0	6	Good
9.3	4.3	74.0	7	Good
		February 18		
8.0	5.0	26	6	Not observed
8.2	4.9	25	5	Not observed
8.3	4.8	18	3	Not observed
8.4	4.7	16	1	Not observed
8.5	4.6	21	4	Not observed
8.6	4.6	17	2	Not observed
8 7	4.6	17	2	Not observed
8.8	4.5	63	7	Not observed
9.0	4.3	66	8	Not observed
		February 21		
6.8	5.0	38	4	Good
7.0	5.0	37	3	Good
7.2	4.9	13	1	Good
7.4	4.7	14	2	Good
7.6	4.6	43	5	Good
7.8	4.6	57	6	Good
8.0	4.4	60	7	Good

Summary of flocculation scores for 0.25 per cent solution, using a volume of 2500 cc.

pH	4.4	4.5	4.6	4.7	4.8	4.9
Average score	5.0	3.2	4.0	2.0	2.0	3.0

1 per cent solution, volume 500 cc.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
		February 18		
cc.		*minutes*		
2.2	5 2	No floc		None
2 3	5 1	3.0	6	Good
2.4	5.0	0.93	4	Good
2.5	4.8	0.33	1	Good
2.7	4.6	0.50	2	Good
2 9	4.5	0.61	3	Good
3.1	4.4	1.40	5	Poor
3.3	4 3	No floc		None
		Repeated February 18		
2.3	5.1	No floc		None
2 4	5.0	2.5	7	Good
2.5	4.9	1.03	5	Good
2.6	4.8	0.7	3	Good
2.7	4.7	0.55	2	Good
2.8	4.6	0.41	1	Good
2.9	4.6	0.7	4	Good
3.0	4.6	2.0	6	Poor
3.1	4.5	60.0	8	Poor
3.2	4.4	No floc		None
		February 21		
1.8	5.1	No floc		None
2.0	4.9	1.26	4	Good
2.1	4.75	0.46	1	Good
2.2	4.6	0.81	2	Good
2.3	4.5	0.83	3	Good
2.4	4.4	1.80	5	Fair
2.5	4.3	No floc		None
		February 25		
2.2	5.1	No floc		None
2.3	4.9	0.4	1	Good
2.4	4.9	0.91	4	Good
2.5	4.7	0.6	2	Good
2.6	4.6	0.93	5	Good
2.7	4.5	0.73	3	Good
2.8	4.4	0.93	6	Good

Summary of flocculation scores of 1 per cent solution, using a volume of 500 cc.

pH	4.4	4.5	4.6	4.7	4.8	4.9
Average score	5.0	3.0	2.8	1.6	2.0	3.5

1 per cent solution, volume 2500 cc.

ACID	pH	TIME OF FLOC	SCORE	SEDIMENTATION OVERNIGHT
February 21				
cc.		*minutes*		
12.0	5.2	No floc		None
13.0	5.0	2.0	4	Good
13.5	4.9	1.0	3	Good
14.0	4.8	0.5	1	Good
14.5	4.6	0.6	2	Good
15.5	4.6	0.5	2	Good
16.0	4.4	59.0	5	Poor
February 25				
12.0	5.2	Overnight	6	Good
12.5	4.9	0.93	4	Good
13.0	4.8	0.6	3	Good
13 5	4.7	0.6	2	Good
14.0	4.6	0.33	1	Good
14.5	4.5	2.0	5	Fair
15.0	4.4	No floc		None
15.5	4.3	No floc		None

Summary of flocculation scores of 1 per cent solution, using a volume of 2500 cc.

pH	4.4	4.5	4.6	4.7	4.8	4.9	5.0
Average score	5.0	5.0	1.5	2.0	3.0	2.6	4.0

Summary of all flocculation scores

pH	4.4	4.5	4.6	4.65	4.7	4.8	4.9	5.0	5.1	5.2
Average score	4.2	3.5	2.7	2.1	2.05	2.4	3.03	2.7	3.0	4.1

These results show that the precipitation of casein from dairy wastes is very satisfactory within the pH limits of 4.9 and4.5. Since flocculation and sedimentation occur through this comparatively wide range of pH values the use of sulphuric acid should prove practicable in plant work.

The method to be used in disposing of the waste from a plant would be as follows: A water-proof tank should be constructed having a capacity somewhat greater than the volume of wash water and other waste which leave the plant daily. While the plant is in operation the combined waste should be delivered into this tank where it is mixed with sulphuric acid in such amount as will produce a red color with methyl red and a deep purple with brom-phenol blue, when small portions are withdrawn and

tested with those indicators. This will keep the pH within the limits of 4.6 and 4.8, or, at the outside, of 4.4 and 5.0. With a very little experience a plant operator of ordinary intelligence should be able accurately to adjust the pH of the waste and acid mixture to the desired value of 4.7. Agitation of some sort would be necessary for a short time to insure complete mixing of the acid and waste. The treated waste is now allowed to remain in the tank overnight when, except for the surface scum, almost complete sedimentation will have occurred.[3] An outlet should be provided for the tank at a sufficient height from the bottom that the supernatant liquid may flow off without disturbing the sediment. The outlet should have a check valve which would prevent lowering the surface of the liquid to that position where the surface scum could flow off. This supernatant liquid could then enter the stream or ditch without causing any considerable nuisance, as is shown by a study of the Relative Stability Values of the raw and treated waste.

Temperature does not affect flocculation or sedimentation, for satisfactory effluents were obtained from samples held at summer temperatures as well as from those which had frozen.

The length of time such a tank could operate without being cleaned would depend upon the concentration of the waste and the temperature. An accumulation of sediment from 1 per cent solutions was held in the laboratory at approximately 23° for eight days and then developed a slight odor. So it is believed that a weekly cleaning and disinfecting of the tank would be satisfactory for the greater part of the year. However during July and August undoubtedly a more frequent cleansing would be necessary, say, either two or three times a week. The ultimate disposal of the casein could be managed in any one of several ways: such as rapid drying and burning; plowing under the soil, or, perhaps, feeding to hogs. However, this last suggestion should be passed upon by an authority on hogs, before being tried.

From the results of the laboratory experiments it can be seen that different amounts of acid were required to produce the same

[3] In a few cases which were observed after standing seven hours, sedimentation was good, indicating that a shorter detention period might be satisfactory.

pH value. This is attributed to the difference in buffer strengths of the various milks used, for the tap water used for all dilutions showed a fairly constant pH value and buffer strength. In plant operation the wash water would always come from the same source and therefore it could be expected to have a farily constant pH value and buffer strength. It is probable that the milk, coming from the same sources would also be more uniform in composition than the different samples which were used in these experiments. Therefore it is anticipated that the amount of acid required to produce pH 4.7 in a known volume of waste could be rather accurately estimated.

Figuring that the volume of waste from a small plant would be 1000 gallons per day, if commercial sulphuric acid of 50° to 60° Baumé were used it would require about 1 pound of acid per day at a cost of from 3 to 5 cents to precipitate the casein.

An attempt was made to study the biochemical oxygen demand of the raw waste, designated as influent, and of the supernatant liquid resulting from precipitation of casein and overnight sedimentation, designated hereafter as effluent. The method used consisted of a determination of the dissolved oxygen present in the sample before and after a period of five days' incubation,—the difference between these two results being the oxygen used by the sample.

In Public Health Bulletin No. 97, 1918, by E. J. Theriault and H. B. Hommon, very exact directions for the application of this method are given. This technic was followed closely, but concordant results with various dilutions could not be obtained. After several unsuccessful attempts, which consumed much time, this method of comparing the influent and effluent was abandoned.

The relative stability of the influent and effluent was studied, using the method given in "Standard Methods of Water Analysis" American Public Health Association(9). "The time required to exhaust the available oxygen in a sample, as indicated by the decolorization of methylene blue at the incidence of putrescence, is a measure of the relative stability." The influent was allowed to stand overnight before incubating so that it would at all times be subjected to the same conditions as the effluent. The temperature of incubation was 20° + or −2°.

In the following table, "volume" = volume of milk solution treated; "per cent" = its percentage concentration; "R.S.I." = relative stability of the influent; "R.S.E." = relative stability of the effluent, and "pH" = hydrogen ion concentration of the effluent.

VOLUME	PER CENT	R.S.I.	R.S.E.	pH
		February 6		
500	0.50	16	80	4.5
			82	4.7
			82	4.8
			82	5.05
		February 8		
500	0.50	21	60	4.6
			70	4.7
			84	4.7
2500	0.50	21	64	4.95
500	0.25	21	70	4.85
2500	0.25	21	84	4.75
			84	4.65
			84	4.5
			84	4.45
		February 12		
500	0.50	14	60	5.1
			84	4.9
			99	4 6
			99	4.4
500	0 25	14	60	5.2
			84	5 0
			88	4 8
2500	0.25	14	95	4.8
			95	4.7
			95	4.6
			95	4.4
			94	4.3
		February 16		
2500	0.25	21	80	4.3
			95	4.35
			95	4.4
			95	4.6
			95	4.7
			95	4.8
			60	5.0

VOLUME	PER CENT	R.S.I.	R.S.E.	pH
February 19				
2500	0.25	30	80	5.0
			97	4.9
			99+	4.8
			92	4.7
			99+	4.6
			99+	4.6
			98	4.6
			99+	4.5
			99+	4.3
500	1.0	16	60	5.0
			60	4.9
			99+	4.7
			98	4.6
			99+	4.6
			97	4.6
February 21				
500	1.0	16	64	4.9
			94	4.75
			80	4.6
			99	4.5
			97	4.4
2500	1.0	16	64	5.0
			68	4.9
			80	4.8
			80	4.6
2500	0.25	16	95	5.0
			97	4.9
			99	4.7
February 26				
500	1.0	16	37	4.9
			40	4.9
			68	4.7
			68	4.6
			97	4.5
			98	4.4
2500	1.0	16	16	5.2
			37	4.9
			44	4.8
			60	4.7
			96	4.6
			98	4.5

SUMMARY

1. Flocculation and sedimentation of the casein in milk wastes is good within the pH limits of 4.5 and 4.9.

2. The supernatant liquid resulting from waste so treated has a high relative stability number.

3. After detaining the treated waste in tanks until good sedimentation has occurred, the supernatant liquid can be discharged into a small stream or ditch without causing a nuisance.

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CLARIFICATION OF MILK FOR CHEESE MAKING[1]

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The clarification of milk has been a common practice in the modern milk plant for a number of years. Recently its application has been advocated in connection with milk to be used in the manufacture of cheddar cheese. The latest report found upon the subject is that given by Fisk and Price (1). These investigators summarize their findings as follows:

> There were 82 cheese made and 150 scorings. The average of the scores shows that the clarified-milk cheese scored 44.948 in flavor and the unclarified 43.666. In body and texture the clarified milk cheese scored 23.858 and the unclarified 23.185. The averages of the total scores were 93.773 for the clarified milk cheese, and 91.908 for the unclarified milk of both good and poor quality was used, and clarification of the milk seemed to improve the quality of the cheese from either. Starter was used in some tests and not in others, showing that clarification improves the cheese whether or not starter is used. The clarifier will sometimes overcome the gas in milk and curd; at other times it will not overcome this gas, but will change it.

Their results indicate an average improvement in the total score of cheese made from clarified milk of 1.865 as compared with cheese made from unclarified milk.

Manufacturing about 400 pounds of cheddar cheese daily, from milk of a poor quality, the Pennsylvania State College Creamery offered an excellent opportunity to study this problem. Work on clarification of this milk as a means of improving the quality of the cheese was started in December, 1922, and was completed in May, 1924.

[1] The data presented in this paper form a part of the thesis of N. A. Hugglar submitted in partial fulfillment of the requirements for the degree of Master of Science at the Pennsylvania State College.

This work was conducted on poor quality and milk of a high quality. The poor quality milk was the milk regularly delivered by the creamery patrons and the high grade milk used was produced by the College Dairy producing milk under certified requirements. It was placed in a large cheese vat thoroughly mixed and a portion removed for clarification. Cheddar cheese was then made from the clarified and unclarified milk. It was placed in the curing rooms for six to eight weeks, then removed and scored by three judges.

An attempt was made to learn if the care given utensils in connection with clarification would bear any relation to the resulting cheese. Milk received by the College Creamery was cared for under the ordinary conditions practiced in the creamery and a series of cheese made under these conditions. Following this experiment a series of cheese were made from the same grade of milk, but extreme care was taken in connection with cleansing all utensils and vats with which the milk came in contact. The vats were sterilized by filling them with boiling water for eight to ten hours before using, also the clarifier parts, the cans and the buckets were carefully sterilized.

CLARIFICATION OF POOR QUALITY MILK FOR CHEESE MAKING

This experiment was divided into two parts. In part 1 milk of a poor quality was handled under ordinary conditions after reaching the creamery. Conditions were not bad but no extreme precautions were made. In part 2 after the milk reached the creamery it was handled with extreme care, all utensils and equipment were sterilized before using. In each part the milk was divided into two portions one half was clarified, the other was not, then both were made into cheese. The results of this experiment are tabulated in table 1.

The results as shown in table 1 do not indicate any improvement in the cheese made from milk of a poor quality handled under practical conditions. However, with extreme care there was an improvement of 1.59 points in total score in favor of clarification. The cheese made from unclarified milk handled under ordinary

conditions had an average score of 86.01 while that made from the same grade of milk and clarified had an average score of 85.22.

It was frequently observed that there was less gas in the clarified milk than in the unclarified milk.

Figure 1 illustrates the only extreme case of gas reduction due to clarification in the manufacture of 82 cheese.

TABLE 1

Quality of cheese manufactured from clarified and unclarified milk of a poor quality, under ordinary and under very sanitary conditions

TRIAL NUMBER	SCORE OF CHEESE UNDER DIFFERENT METHODS OF HANDLING MILK			
	Ordinary		Very sanitary	
	Unclarified	Clarified	Unclarified	Clarified
1	80.0	77.0	84.0	84.0
2	90.0	89.0	86.33	98.5
3	88.0	87.0	88.66	90.0
4	86.0	86.0	84.0	89.66
5	85.16	83.4	80.83	87.5
6	87.0	85.5	92.0	87.0
7	82.75	87.33	83.0	83.0
8	88.5	83.8		
9	88.0	88.5		
10	89.0	87.7		
11	86.5	85.75		
12	82.5	81.75		
Average......	86.01	85.22	85.55	87.14

CLARIFICATION OF A HIGH GRADE MILK FOR CHEESE MAKING

The first experiment was repeated in every detail with the exception of the grade of milk used in the manufacture of the cheese. The results are tabulated in table 2.

The results shown in table 2 clearly indicate that the cheese made under ordinary methods show an improvement of 2.1 points due to clarification while that made under sanitary conditions indicate an improvement of 2.87 points.

The average flavor score of cheese made from the clarified milk handled under practical conditions shows an improvement of 1 point over the unclarified milk and 1.16 points improvement on body and texture. Where the milk was handled under

extreme sanitary conditions the improvement due to clarification was 0.83 point in flavor and 1.66 points in body and texture. Figure 2 illustrates a representative group of the cheese made in this experiment.

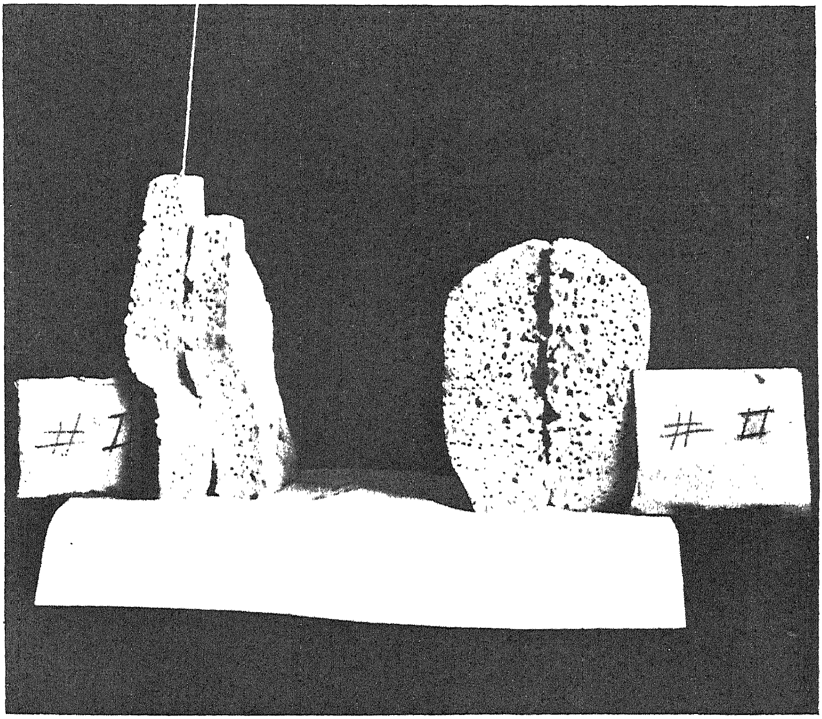

FIG. 1. (1) CURD PRODUCED FROM CLARIFIED MILK; (2) CURD PRODUCED FROM UNCLARIFIED MILK

The average total score of 82 cheese shows an improvement of 1.03 in favor of the clarified cheese. The score for the cheese was as follows:

41 clarified	87.58
41 unclarified	86.55

Under present market conditions the above cheese would sell for the same price. It would therefore be doubtful, whether the process of clarification can be justified in the average cheese factory.

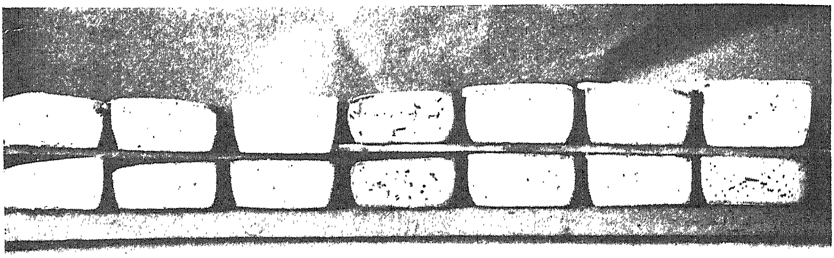

FIG. 2. A REPRESENTATIVE GROUP OF CHEESE MANUFACTURE IN EXPERIMENT 2 FROM CLARIFIED AND UNCLARIFIED MILK

Top row, unclarified; bottom row, clarified

TABLE 2

Quality of cheese manufactured from a high grade of milk clarified and unclarified and handled under ordinary and under very sanitary conditions

TRIAL NUMBER	Score of cheese under different methods of handling milk: Unclarified, Flavor	Unclarified, Body	Unclarified, Color	Unclarified, Total	Clarified, Flavor	Clarified, Body	Clarified, Color	Clarified, Total
				Ordinary				
1	25.0	32.0	8.0	85.0	25.0	35.0	8.0	88.0
2	24.0	34.0	8.0	86.0	24.0	35.0	9.0	88.0
3	22.0	32.0	9.0	82.0	24.0	35.0	8.0	87.0
4	20.0	32.0	9.0	81.0	24.0	35.0	9.0	88.0
5	26.0	35.0	9.0	90.0	24.0	33.0	9.0	86.0
6	24.0	33.0	8.0	85.0	26.0	32.0	9.0	87.0
Average..	23.5	33.0	8.33	84.8	24.5	34.16	8.67	87.33
				Very sanitary				
1	24.66	34.0	9.33	88.0	26.33	36.0	10.0	92.33
2	24.66	31.33	9.0	85.0	26.66	36.33	10.0	93.0
3	23.66	31.33	9.33	84.33	23.33	31.33	9.0	83.66
4	24.0	33.0	9.0	86.0	24.0	32.66	9.0	85.66
5								
6								
Average..	24.5	32.42	9.16	85.83	25.08	34.08	9.5	88.66

SUMMARY

A total of 82 cheese were made in this experiment. Clarification of a poor quality of milk under ordinary conditions showed no improvement in the score of cheese.

Clarification of poor quality milk handled under strictly sanitary conditions resulted in an improvement of 1.59 points in the score of the cheese.

Clarification of a high quality milk under ordinary conditions resulted in an improvement of 2.53 points.

Clarification of a high quality milk under strictly sanitary conditions resulted in an improvement of 2.83 points in the total score of the cheese.

On the average clarification resulted in an improvement of 1.03 points in total score.

The writers wish to express their thanks to C. W. Fryhofer formerly of the United States Department of Agriculture, Dairy Division, for his assistance in scoring a number of the cheese used in this experiment.

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REVIEW OF FOREIGN DAIRY LITERATURE

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REISS, VALBERG. *Use of saltpeter in cheese making.* (Milchwirtschaftl. Zentrabl. 1922. 51. Jahrg. H. 6. S. 68–69.)

The action of saltpeter probably consists in giving up its soluble acid to bacteria that need it for their development, while otherwise they would have to attack the milk sugar and other ingredients whereby compounds and gases detrimental to the taste of the cheese may result. The amount of saltpeter must be regulated according to the quality of milk, kind of cheese, acidity, storage and later holding temperature no matter if we deal with pasteurized or raw milk. Experience there is of great importance. Too large amounts hurt the cheese, because it forms in that case a flaming red colored mass with an unclean taste and the ripening period is prolonged. If fermenting feeds or green feed of vetch, alfalfa, etc., are used saltpeter may be used up to 30 to 60 grams per 100 kgm. of milk. More may be used for Gouda, Edam and home made cheese types than for higher acid, much stirred cheese (cheddar, pastor) or for late warmed (large holed cheeses) or for pasteurized milk cheese or for types of cheeses in which cultures of rod shaped lactic acid bacteria are used (Swiss). Since the latter retard the fermentations in the cheese by the acid formed and thus act in this direction like saltpeter this amount must be decreased in the same ratio as the amount of pure culture is increased. In pasteurization of cheese milk the bacteria causing a gassy fermentation are partly killed and such milk requires only half as much saltpeter as unpasteurized milk. With a low storage temperature less saltpeter is used than with a high one. The use of saltpeter makes storage at a higher temperature possible whereby curing is hastened without injury to the cheese. Saltpeter must be added to the milk with thorough stirring before setting. If a 2-liter solution contains 1 kg. saltpeter, then use twice as many cc. as the grams of saltpeter that are desired. The saltpeter should be dissolved in warm water and filtered; KNO_3 or $NaNO_3$ may be used. Matouschek (Vienna).

MUELLER, A. *Experimentation keeping milk sweet by adding small amounts of hydrogen peroxide.* (Milchwirtsch. Zentralbl. Jahr. 51. 1922. S 25–29, 37–39, 49–53, 61–64.)

The bactericidal action of H_2O_2 is due to the undecomposed H_2O_2 itself and not the O which is freed during the breaking up by catalase. Catalase actions must therefore be disregarded in the addition of this medium. The author conducted experiments to ascertain whether it is possible to keep milk fresh without changing the taste of the milk by adding H_2O_2 after inactivating the catalase by heat. The milk came from a cow stable in Berlin-Friedenau. To inactivate the catalase heating to 70 to 71° for one-half hour was practiced; then cooling to the experimental temperature, making the H_2O_2 additions, filling into not sterile Erlenmeyer flasks and holding in the dark. The milk kept with the addition of 0.1 to 0.15 per cent H_2O_2 three and four times as long as milk which was just pasteurized one-half hour at 70°C. At first the taste of the milk was somewhat affected, later not at all. The same milk without H_2O_2 spoiled regularly in twenty-four to forty-eight hours. Also milk which was exposed to air contamination in cooling kept with the given amount of H_2O_2 added at least twenty-four hours longer than the H_2O_2 free control milk. In dairies good results will be obtained, if the air contamination is avoided during cooling by using a light protective guard around the cooler and if the splitting of the H_2O_2 by catalase caused by rust is avoided by using well tinned cans. Matouschek (Vienna).

MAZZEO, MARIO. *On the mechanism of the coagulation of milk produced by bacteria.* (Pathologica An., 14, 1922, p 162–171.)

In the experiments the dependence of the force and speed of the coagulation of milk upon the acid content of the milk came to light. Bac. bifidus alone acts through acid formation. Enterococcus and also Pneumococcus act thus and also by means of a coagulating enzyme.

Rona, P., and Gabbe, E. On the influence of calcium on the rennet coagulation of milk (Biochem. Ztschr., vol. 134, 1922, p. 39).

The authors came to the following conclusions:

1. The action of calcium on the rennet coagulation of milk was studied by adding calcium chloride to the ten to thirty times diluted and with rennet mixed milk during different stages of the enzyme activity and by noting the time until the beginning of coagulation. The hydrogen ion concentration of the milk was regulated by means of acetate buffer.

2. The progress of the enzyme action was analyzed by determining the temperature at which the milk at the different stages may be caused to coagulate. Considering the Ca-content, degree of dilution and pH value the temperature of coagulation proved to be characteristic and determinable with sufficient accuracy for a certain stage during the change of the casein into paracasein by the enzyme.

3. By means of the coagulation temperature of mixtures of coagulating and rennet-free milk it is possible to replace the coagulation temperatures determined in the course of a certain coagulation experiment by conversion figures which show how much of the existing casein was changed into paracasein by the enzyme.

4. If the calcium chloride is added at the beginning of the enzyme action the time until the beginning of coagulation is longer the higher the calcium content of the solution is. The experiments to analyze the enzyme action show that low calcium concentrations favor the enzyme action, while the higher ones retard it.

5. If the calcium chloride is not added until during the enzyme action then a prolongation of the time required for coagulation is regularly noticed, which varies with the time of adding the calcium and the calcium concentration. This prolongation of the time of coagulation occurs also with such $CaCl_2$ concentrations which when added at the beginning of the enzyme action hasten the action. This action of the calcium when added later may be explained by a retardation of the enzyme action and by changes in the precipitability of the paracasein by the calcium.

6. The change of the casein into paracasein is complete only at a pH value of 6.0 to 6.4 which is the optimum for precipitation of the paracasein calcium. At a higher hydrogen ion concentration coagulation takes place while the casein change is incomplete. Heuss (Berlin).

Stokoe, U. *Rancidity of butter and margarine.* (Chem. Umschau, Bd. 28, 1921, S. 132.)

The rancidity of water-free fats is not due to the activity of microorganisms; the author inoculated several fats and oils with a culture from rancid margarine and after two weeks found only a very slight increase in the free fatty acid and no changes in odor and taste. The rancidity of margarine falls into three groups:

1. Tallowy odor and taste, no change in color, caused by an oxidation process in which glycerin already split off is oxidized to glycollic acid.

2. Aromatic odor, disagreeable taste, only in margarine made from cocoanut oil or palm kernel oil. Penicillium glaucum produces the odoriferous substances.

3. Slight changes in odor and taste; Aspergillus produces gray or bluish black coloring, Bacillus prodigiosus red coloring. Many oils and fats of commerce are filled with molds and other spore forming organisms. Therefore it is necessary to examine these carefully and to keep them pure.

American Dairy Science Association Meets at Milwaukee

The Wisconsin hotel will be headquarters for the A. D. S. A. at Milwaukee. Write E. A. Rolph, manager of the hotel, for reservations, mentioning the fact that you are a member of the association.

All the meetings of the American Dairy Science Association will be held in the Milwaukee auditorium on Monday, September 29, 1924. The general session starting at 9:00 a.m. followed by the meetings of the production, manufacturing, and extension sections at 1:30 p.m., and the advanced registry section at 7:30 p.m.

The general session of the American Dairy Science Association will be addressed by E. D. Ball, in charge of the Scientific department of the U. S. Department of Agriculture, by L. A. Rogers of the dairy bureau and by the Editor of the Journal of Dairy Science, Dean H. L. Russell and Chief Larson will be the speakers at the banquet. The program of the Production section is as follows:

1:30 P.M., SEPTEMBER 29, 1924

Call to order by Chairman........................J. J. Hooper
Reading of minutes of last meeting, Secretary.....J. A. Gamble
Address: "Twenty Years of Progress in Animal Nutrition"
Dr. E. B. Hart
Report of Committee on National Students Judging Contest
W. W. Swett, *Chairman*
Report of Southern Contest............J. J. Hooper, *Chairman*
Address: "Some Observations on the Relation of Conformation to Production in Dairy Cattle"..............R. R. Graves

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The program of the other sections will be available later

A STUDY OF THE RELATION BETWEEN FEED CONSUMPTION AND MILK SECRETION

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Received for publication August 25, 1924

While many yearly milk production records are available which permit the study of the normal course of milk secretion during the lactation period, records of feed consumption during the course of the lactation period are very limited in number. Studies showing the relation between total feed consumption and total milk production are the basis of the modern feeding standards for dairy cattle, but the relation between feed consumption and milk secretion during the course of the lactation period has not been determined.

Study (1) of the characteristics of the yearly milk secretion curves of the various breeds of dairy cattle and a number of groups of cows within certain breeds indicate that under favorable conditions of feeding and management the factors controlling the rate of increase in milk secretion during the early part of the lactation period and the rate of decline following the period of maximum production appear to be inherited characteristics governing the mechanism of milk secretion.

The object of this paper is to present data showing the characteristics of the feed consumption curves during the course of the lactation period and their relation to milk secretion and change of live weight.

SOURCE OF DATA

Through the kindness of C. M. Long, Secretary of the Illinois Holstein Friesian Association and W. H. Dressel in charge of the Illinois Testing Plant at Dixon, Illinois, the writer secured for study and analysis the complete feed, milk, live weight, age,

and breeding records of approximately 50 Holstein-Friesian cows on yearly test. Grateful acknowledgment to these gentlemen for making this study possible is given.

FEED AND MANAGEMENT OF TEST COWS

The cows at the Illinois testing plant were provided with individual box stalls. As each cow was kept isolated so as to prevent spread of disease it was impossible to let the cows out to pasture. A limited amount of exercise, however, was provided. In many cases the cows were brought to the plant several months previous to the time of calving in order to be properly fitted for the test. While feed records were kept during this period they were not studied.

Complete accurate records were kept of the concentrates and roughage fed to each individual cow. The daily milk production was recorded as required for Advanced Registry. The weight of each cow was carefully estimated by the feeder and herdsman each month. The date of service was also recorded. In general it may be said that the cows were kept under the best possible conditions and every effort was made to secure maximum milk production from each cow.

The records should therefore be (as nearly as it is possible to secure) the actual inherited capacity for production. The course of milk secretion should also represent the inherited rate of increase in milk production, the amount of production at the maximum, and the rate of decline of milk secretion or the persistency of production.

As feed was provided up to the maximum of consumption without throwing the cow off feed, the feed records should indicate the rate of increase of feed consumption, the time of maximum consumption, and the rate of decline of the appetite as milk secretion declines.

FEEDS USED AT TESTING PLANT

In table 1 a list of all feeds used at the testing plant are included. The content of digestible nutrients in the several feeds were taken from the appendix table of "Feeds and Feeding"

by Henry and Morrison. The degestible nutrients in the commercial mixed feeds were secured from the manufacturer. This list includes most of the popular feeds fed to test cows in the mid-west.

TABLE 1

Feeds used at the testing plant

NAME OF FEED	CRUDE PROTEIN	CARBO-HYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS
Ground corn (dent grade 2)	7.1	74.6	81.7
Corn germ meal	16.5	66.0	82.5
Corn gluten meal	30.2	53.8	84.0
Cottonseed meal	37.0	41.2	78.2
Distillers grains (from corn)	22.4	66.5	88.9
Hominy feed	7.0	77.6	84.6
Linseed meal (O. P.)	30.2	47.7	77.9
Oat meal (rolled oats)	12.8	70.4	83.2
Oats, ground	9.7	60.7	70.4
Soybean oil meal	39.7	44.8	84.5
Cocoanut meal (low in fat)	18.8	60.2	79.0
Wheat bran (all analyses)	12.5	48.4	60.9
Wheat middlings (shorts)	13.4	55.9	69.3
Alfalfa hay (all analyses)	10.6	41.0	51.6
Beet pulp	4.6	67.0	71.6
Beets, common	0.9	9.3	10.2
Corn silage	1.1	16.6	17.7
Unicorn	21.3	61.0	82.3
Purina cow chow	20.2	51.5	71.7
Champion (estimated)	20.2	54.3	74.5

RELATION BETWEEN THE INITIAL INCREASE IN FOOD CONSUMPTION AND MILK SECRETION

From the daily feed records, the amount of digestible crude protein, carbohydrate equivalent (fat $\times$ 2.25 + carbohydrate) and total digestible nutrients consumed by each cow was determined. The average daily consumption of digestible nutrients for the first sixty days is presented in table 2. In figure 1 is presented the relation between feed consumption and milk secretion during this period. It will be seen that there is a gradual increase in the nutrients received throughout the entire sixty-day period. In fact there is a slight increase until the

fourth month in the average daily consumption of nutrients. In comparison to feed consumption, milk secretion increases rapidly reaching a maximum about thirty-five days after parturi-

TABLE 2

Average daily feed consumption

DAYS AFTER CALVING	DIGESTIBLE CRUDE PROTEIN	CARBO-HYDRATE EQUIVA-LENT	TOTAL DIGESTIBLE NUTRIENTS	DAYS AFTER CALVING	DIGESTIBLE CRUDE PROTEIN	CARBO-HYDRATE EQUIVA-LENT	TOTAL DIGESTIBLE NUTRIENTS
	pounds	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
1	3.75	18.38	22.13	31	5.37	22.75	28.12
2	3.82	18.65	22.47	32	5.39	22.92	28.31
3	3.90	18.86	22.76	33	5.42	22.99	28.41
4	3.95	18.97	22.92	34	5.43	23.24	28.67
5	4.11	19.67	23.78	35	5.46	23.26	28.72
6	4.21	19.89	24.10	36	5.47	23.11	28.58
7	4.29	20.14	24.43	37	5.52	23.18	28.70
8	4.29	20.11	24.40	38	5.56	23.30	28.86
9	4.42	20.01	24.43	39	5.62	23.44	29.06
10	4.53	20.08	24.61	40	5.62	22.97	28.59
11	4.56	20.13	24.69	41	5.64	23.52	29.16
12	4.64	20.28	24.92	42	5.66	23.54	29.20
13	4.68	20.43	25.11	43	5.66	23.50	29.16
14	4.76	20.68	25.44	44	5.66	23.51	29.17
15	4.81	20.82	25.63	45	5.66	23.55	29.21
16	4.80	20.89	25.69	46	5.68	23.57	29.25
17	4.96	21.20	26.16	47	5.72	23.57	29.29
18	5.16	21.16	26.32	48	5.76	23.68	29.44
19	5.18	21.10	26.28	49	5.72	23.61	29.33
20	5.24	21.83	27.07	50	5.75	23.74	29.49
21	5.26	21.95	27.21	51	5.75	23.73	29.48
22	5.27	21.99	27.26	52	5.75	23.75	29.51
23	5.26	22.49	27.75	53	5.74	23.77	29.51
24	5.38	22.48	27.86	54	5.76	23.82	29.58
25	5.29	22.08	27.37	55	5.76	23.85	29.61
26	5.33	22.23	27.56	56	5.77	23.87	29.64
27	5.34	22.25	27.59	57	5.77	23.87	29.64
28	5.34	22.30	27.64	58	5.78	23.92	29.70
29	5.36	22.36	27.72	59	5.80	23.95	29.75
30	5.35	22.42	27.77	60	5.80	24.04	29.84

tion. This milk is evidently secreted, in the absence of a sufficient intake, at the expense of the body, causing a loss of live weight. It is evident from these facts that the initial rise in milk secretion may be limited by the condition of the cow

at the time of calving as well as by the frequency of milking (1) and the inherited factors which regulate or limit the rate of increase of secretion. It is important, therefore, in studying

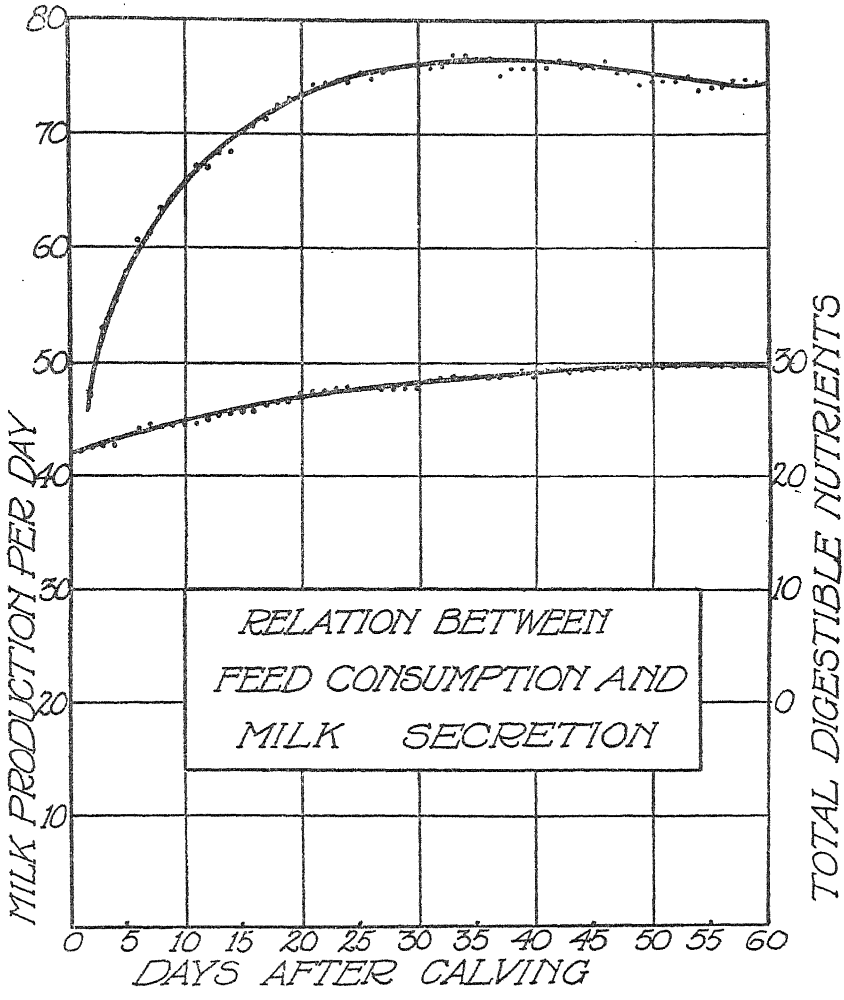

FIG. 1. RELATION BETWEEN FEED CONSUMPTION AND MILK SECRETION DURING THE FIRST SIXTY DAYS AFTER CALVING

While milk secretion increases rapidly reaching a maximum about thirty-five days after calving, there is a gradual increase in the nutrients consumed throughout the entire sixty-day period.

the initial rise of milk secretion, that the various factors which may limit or retard the full demonstration of the inherited qualities be understood and controlled as far as possible.

THE RATE OF DECLINE OF FOOD CONSUMPTION AND MILK SECRETION

After milk secretion reaches a maximum there follows a gradual decline. The law governing the decline of milk secretion with the advance of the period of lactation has been shown by work at this Station (1, 2). This law may be expressed by

TABLE 3

Summary of monthly milk production

MONTH OF LACTATION	NUMBER OF COWS	AVERAGE MONTHLY PRODUCTION OF MILK	AVERAGE NUMBER DAYS IN MILK	AVERAGE DAILY MILK PRODUCTION	PERCENTAGE OF PREVIOUS MONTH'S PRODUCTION
		pounds		*pounds*	
1	49	1,028.2	15.5	66.3	
2	49	2,436.1	30.2	80.7	
3	49	2,222.9	30.3	73.4	91.1
4	49	2,084.7	29.8	70.0	95.3
5	49	1,957.9	30.5	64.2	91.7
6	49	1,846.1	30.4	60.7	94.6
7	49	1,690.7	30.4	55.6	91.5
8	49	1,624.7	30.6	53.1	95.5
9	49	1,528.7	30.3	50.5	95.1
10	49	1,460.7	30.6	47.7	94.4
11	49	1,350.7	30.0	45.0	94.3
12	48	1,266.2	29.3	43.2	96.0
13	39	721.3	16.4	43.9	

saying that each month's production after the time of maximum is a constant percentage of the preceding months' production. Pregnancy (3), season (4), and other factors of feeding and management, however, cause minor exceptions to this law. The monthly rate of decline of milk secretion of these cows is shown in table 3. The average rate of decline thoughtout the lactation period is 93.95 per cent of the previous month's production. This factor indicates the persistency of the cows included.

How does the decline in feed consumption compare with the decline in milk secretion? As shown by table 4, feed consumption

also appears to follow a definite law of decline. It may be expressed as follows: After maximum feed consumption is

TABLE 4

Average daily feed consumption by months

MONTH OF LACTATION	DIGESTIBLE CRUDE PROTEIN	CARBO-HYDRATE EQUIVALENT	TOTAL DIGESTIBLE NUTRIENTS	NUTRITIVE RATIO	PERCENTAGE PREVIOUS MONTH'S FEED CONSUMPTION
	pounds	*pounds*	*pounds*		
1	4.63	20.38	25.01	1:4.4	
2	5.41	22.76	28.17	4.2	
3	5.69	23.36	29.05	4.1	
4	5.83	24.05	29.88	4.1	
5	5.92	23.55	29.47	3.9	98.62
6	5.81	23.28	29.09	4.0	98.71
7	5.81	22.55	28.36	3.8	97.49
8	5.77	22.39	28.16	3.8	99.29
9	5.59	21.90	27.49	3.9	97.62
10	5.57	21.32	26.89	3.8	97.81
11	5.29	20.93	26.22	3.9	97.51
12	5.17	20.22	25.39	3.9	96.83

TABLE 5

Summary of monthly change in live weight

MONTH OF LACTATION	NUMBER OF COWS	AVERAGE LIVE WEIGHT
		pounds
1	45	1,370
2	46	1,321
3	46	1,296
4	46	1,300
5	46	1,304
6	46	1,315
7	46	1,314
8	46	1,311
9	46	1,319
10	46	1,338
11	46	1,356
12	45	1,384
13	43	1,443

reached, each month's food consumption is a constant percentage of the preceding month's food consumption. The rate of decline of the appetite, indicated by daily feed consumption is slower

than the rate of decline of milk secretion. The average rate of decline of feed consumption is 97.98 per cent of the previous month's consumption.

With a physiological condition prevailing in which the feed consumed contains an excess of nutrients needed for milk secretion, the surplus must be stored as an increase in body weight. This is in fact what happened as shown by table 5.

These data are therefore taken to indicate that under optimum conditions of feeding and management, the curve of milk secretion is regulated by certain inherited factors governing the initial rise of milk secretion, the volume of secretion at the maximum, and the persistence or rate of decline of milk secretion. An excess of feed is merely stored as an increase in body weight. The futility of over-feeding to increase milk secretion is thus shown.

Diary cows whose inheritance for milk production is great may be limited by the lack of capacity for feed consumption. In that event, the decline or persistency in milk production will parallel the decline of feed consumption and the animal will be in thin flesh at the close of the lactation period. Such an animal is said to have "dairy temperament." On the other hand, in animals whose inheritance for milk production or "persistency" is less, the capacity for feed consumption is greater than the need of nutrients for milk production and they will use the surplus for an increase in body weight.

The animals included in the study on the average consumed feed slightly in excess of their capacity for milk production. The limiting factor in their inheritance for milk production was in lacking persistency of milk production rather than a lack of capacity for the consumption of feed.

NUTRIENTS REQUIRED FOR MILK PRODUCTION

There has been considerable discussion among feeders of test cows as to the changes which should be made in the relation between the digestible protein and the carbohydrate equivalent (the nutritive ratio) during the lactation period. This relation

during the course of the lactation period for the cows studies is given in table 4. It will be noted that the nutritive ratio was decreased slightly during the first six months (from 1:4.4 to 3.9) and then was held fairly constant during the rest of the lactation period.

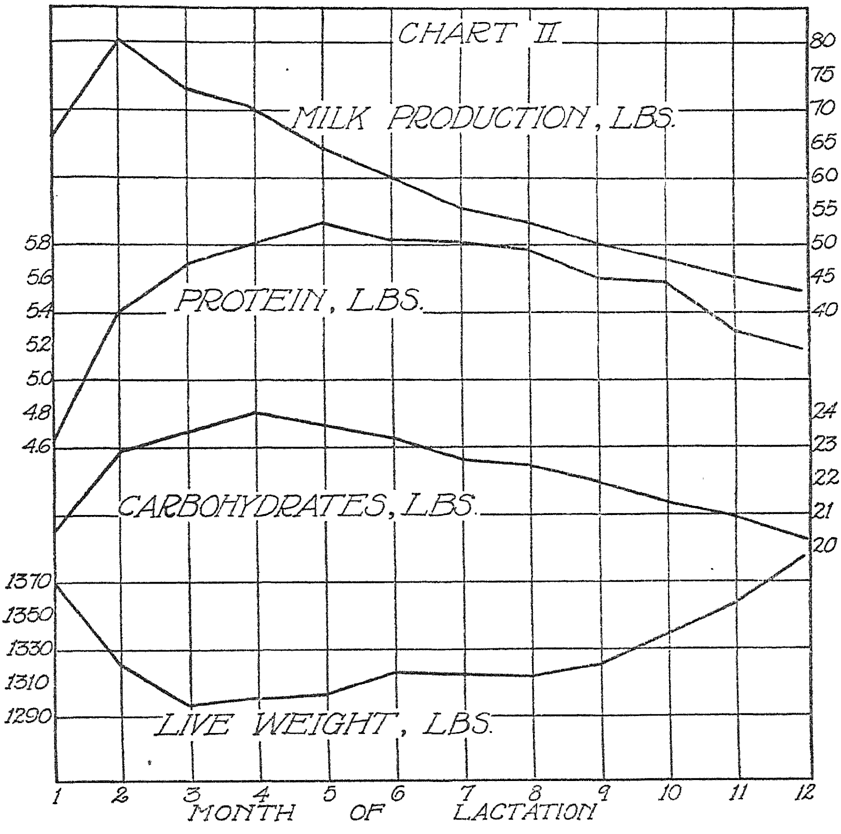

FIG. 2. THE CHANGE IN MILK PRODUCTION, FEED CONSUMPTION, AND LIVE WEIGHT DURING THE COURSE OF THE LACTATION PERIOD

In figure 2 the average daily milk production during the lactation period is compared with digestible protein and carbohydrate equivalent consumption. This shows the nutrients consumed to produce the various amounts of milk during the course of the lactation period.

By subtracting the requirements for maintenance prescribed by the Haecker Standard (0.7 pounds digestible crude protein and 7.925 pounds total digestible nutrints per 1000 pounds live weight) from the consumption of the various nutrients and dividing it by the average daily milk yield, the requirement per pound of milk during each month of lactation was determined for 3.5 per cent milk. To make the data applicable to cows producing milk of various fat content the Haecker feeding standard was used as a guide because it is based on extensive

TABLE 6

Feeding standard for cows on test

ONTH OF LACTATION	PER CENT FAT 2.5		PER CENT FAT 3.5		PER CENT FAT 4.5		PER CENT FAT 5.5		PER CENT FAT 6.5	
	Protein	Carbo-hydrate equivalent	Protein	Carbo-hydrate equivalent	Protein	Carbo-hydrate equivalent	Protein	Carbo-hydrate equivalent	Protein	Carbo-hydrate equivalent
1	0.0508	0.126	0.0553	0.158	0.0643	0.189	0.0722	0.216	0.0813	0.245
2	0.0510	0.131	0.0555	0.164	0.0646	0.196	0.0725	0.224	0.0815	0.254
3	0.0598	0.152	0.0651	0.191	0.0757	0.228	0.0850	0.261	0.0957	0.296
4	0.0646	0.166	0.0703	0.209	0.0818	0.250	0.0918	0.285	0.1033	0.324
5	0.0715	0.175	0.0779	0.220	0.0906	0.263	0.1017	0.301	0.1145	0.341
6	0.0739	0.181	0.0805	0.227	0.0936	0.271	0.1051	0.310	0.1183	0.351
7	0.0807	0.187	0.0879	0.235	0.1022	0.281	0.1148	0.321	0.1292	0.364
8	0.0808	0.193	0.0880	0.243	0.1024	0.290	0.1149	0.332	0.1293	0.376
9	0.0849	0.195	0.0924	0.245	0.1075	0.293	0.1207	0.335	0.1358	0.379
10	0.0890	0.195	0.0969	0.245	0.1127	0.293	0.1266	0.335	0.1424	0.377
11	0.0890	0.197	0.0969	0.247	0.1127	0.295	0.1266	0.337	0.1424	0.382
12	0.0893	0.189	0.0972	0.237	0.1131	0.283	0.1270	0.324	0.1428	0.367

data showing the relation between the fat content of milk and its feed requirement for production. The results are shown in table 6.

It is generally believed that the feed cost of milk production under official test conditions is greater than the production under ordinary care. In other words, it is thought the economic law of diminishing returns begin to operative with test cows as milk production increases. Unquestionably this is true in those cases where cows are fed above their inherited capacity for milk production. But until that point is reached it was

found that the milk produced per pound of grain increased as the average yearly milk production increased. The results are shown in tables 7 and 8.

TABLE 7

Relation between yearly milk production and feed consumption

NUMBER OF COWS	MILK	FAT	GRAIN	HAY	BEET PULP	SILAGE
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
4	15,260	541	5,579	4,143	1,686	8,672
10	16,996	576	5,892	4,107	1,541	8,556
7	19,310	640	6,624	4,409	1,794	8,977
7	20,847	689	6,828	4,279	1,722	8,964
10	22,833	733	6,874	4,259	1,767	8,870
4	24,808	768	6,649	4,164	1,612	8,671
4	26,605	830	7,739	4,400	1,781	9,226

TABLE 8

Milk produced per pound of feed

NUMBER OF COWS	MILK	FAT	GRAIN	HAY	BEET PULP	SILAGE
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
4	15,260	541	2.73	3.70	9.04	1.75
10	16,995	576	2.88	4.13	11.03	1.98
7	19,310	640	2.91	4.37	10.70	2.15
7	20,487	689	3.05	4.87	12.00	2.32
10	22,833	733	3.32	5.36	12.90	2.57
4	24,808	768	3.69	5.90	15.20	2.83
4	26,605	830	3.43	6.04	14.90	2.88

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THE CORRELATION BETWEEN CHANGES IN AGE AND MILK PRODUCTION OF DAIRY COWS UNDER OTHER THAN OFFICIAL TESTING CONDITIONS[1]

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Previous investigations have shown the correlation that exists between the changes in age and milk production of dairy cows when the cows are kept under official testing conditions. Many cattle breeders have wondered whether or not the results of those investigations could be applied with accuracy to conditions such as those under which a cow is not being fed and managed for an official record.

It is important that the truth be known concerning the correlation of changes in age and milk production of cows kept under other than official test conditions, since a very small percentage of the dairy cattle of the country are tested for official record. For these cows without official test, there is a need for age correction factors, such that can be used with the immature record of a young cow so as to predict the probable mature production of that animal. With the use of such factors the breeders can tell how profitable the young cow will be when she becomes mature, with at least a fair degree of accuracy.

In order to determine age correction factors for other than official test conditions, the author has studied records from the experiment station herds of the following colleges and universities: University of Minnesota (1), Cornell University (2), University of Nebraska (3), University of Vermont (4), Storrs Connecticut Station (5), University of Missouri (6), Oregon Agricultural

[1] The material for this article is taken from work preliminary to a thesis by R. S. Clark, for the degree of Master of Science at the University of Minnesota.

College (7), University of Wisconsin (8), University of Illinois (9), University of Maryland (10), and the Pennsylvania State College (11).

METHODS OF HANDLING DATA

In collecting the data from which the age correction factors were to be calculated, all irregularities were noted in so far as the records available showed. No records immediately following abortions were used.

The age at which a cow freshened was calculated from the date of birth to the date on which the calf was born. The record was tabulated as of the age to which it was closest. The considerable number of records used should eliminate the error that would result from placing some records under a certain age merely because of the margin of a few days.

No records were included in the age correction study from cows having less than two yearly records. None were used that were made with more than two milkings daily in so far as the records showed. The season at which a cow freshens affect somewhat the percentage of fat in the milk and it may have some effect on the quantity as well.

No correction factors have been calculated by previous workers to equalize this influence and this investigation does not include a determination of such factors. It would be difficult to work out factors for the influence of season of freshening. It would also be hard to apply those factors. A factor for each day would need to be determined so as to cover every possible date of freshening.

The length of gestation period during the time of milking affects milk production. The herds studied were handled in a manner representative of good herd management. The numbers involved should avoid any considerable error on this point. In cases where a cow milked for more than a year in one lactation period, the record for the first 365 days only, was used.

While no information is at hand regarding the details of the feeding practice in the several herds supplying data, it is believed

that these conditions are reasonably uniform and that no special errors are introduced by such differences as do exist.

To measure the mathematical reliability of the data, the standard deviations, coefficients of variability and their probable errors were calculated. Standard formulae were used in these calculations (12).

The data for each of the four breeds studied were tabulated separately. Table 1 shows the division of the material among the Holstein, Jersey, Guernsey, and Ayrshire Breeds.

TABLE 1

Summary of records

Age correction factors to tenth year*

BREED	NUMBER OF RECORDS	NUMBER OF COWS
Holstein	280	77
Jersey	632	189
Guernsey	540	139
Ayrshire	231	70

* The number of records in the ages beyond ten years was so limited in most breeds that those ages were merely tabulated and were not given statistical consideration. Ten years was a satisfactory age at which to conclude the analysis since the point of mature production was found to occur prior to that age for every breed.

DISCUSSION OF DATA

Chart 1 shows in a visual way the results of the investigation. The calculated curve was obtained by the use of the "Star Point Method" (13). The formula for this method is,

$$y = x \text{ plus } bx \text{ plus } cx^2 \text{ plus } dx^3 \; . \; . \; . \; . \text{ etc.}$$

In this formula, $y =$ milk yield in pounds and $x =$ age in years as applied to these figures. The chart also shows the plotting of the observed data.

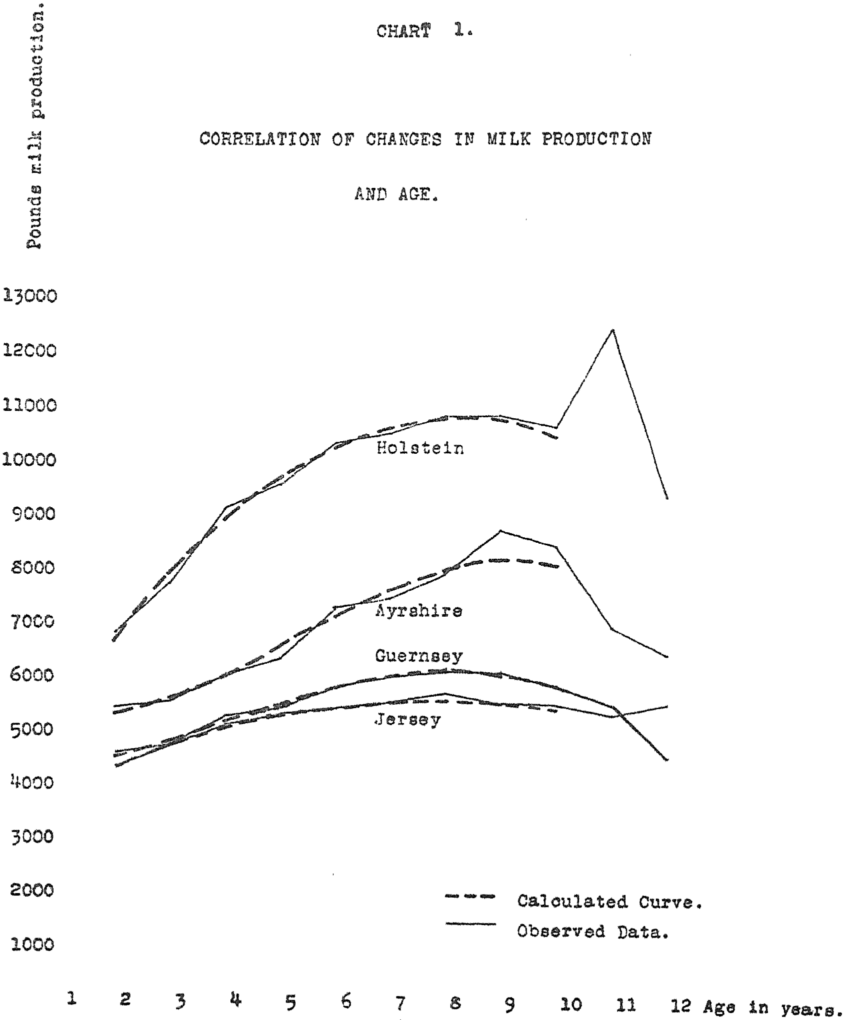
From the chart it can be seen that the point of maximum production is about eight years of age for the Holstein, Jersey, and Guernsey breeds. For the Ayrshires this point is nine years.

Even with experiment station herds there was some discarding of low producers after one or two records have been placed on

CHART 1.

CORRELATION OF CHANGES IN MILK PRODUCTION

AND AGE.



each cow. Allowance must be made for this fact when using these figures. There is however, much less selection of the population in the experiment station herds, than in the case of Advanced Registry Cows.

Table 2 includes the figures from which the observed and calculated curves were charted.

In table 3, a comparison is made between the maturity represented by the ages studied in this investigation and the results

TABLE 2

Age correction data

Calculated and observed data arranged by breeds

CLASS MID POINTS	HOLSTEIN			JERSEY			GUERNSEY			AYRSHIRE		
	Number of cows	Milk		Number of cows	Milk		Number of cows	Milk		Number of cows	Milk	
		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated
years		*pounds*	*pounds*		*pounds*	*pounds*		*pounds*	*pounds*		*pounds*	*pounds*
2	35	7047.7	6881.1	62	4591.7	4619.0	102	4837.2	4750.0	22	5666.3	5510.0
3	50	7949.0	8110.6	93	4998.5	4962.5	110	4996.6	5050.5	41	5774.4	5821.3
4	52	9311.6	9106.9	84	5342.9	5268.6	83	5500.1	5391.4	45	6287.1	6276.8
5	42	9767.2	9900.0	86	5555.9	5537.4	69	5688.1	5700.0	36	6595.0	6800.0
6	29	10530.5	10479.9	78	5693.2	5696.8	53	6066.3	6052.4	23	7526.6	7354.4
7	24	10722.5	10846.6	77	5770.1	5783.0	46	6281.4	6280.7	25	7702.9	7843.2
8	22	11092.1	11000.1	58	5908.6	5795.8	35	6346.7	6400.0	16	8156.6	8200.0
9	17	11098.6	10940.4	54	5756.9	5745.3	24	6313.4	6278.0	12	8968.1	8387.6
10	9	10894.1	10667.5	40	5717.0	5601.5	18	6083.4	6100.0	11	8688.1	8300.0
11	9	12691.1		38	5513.0		14	5742.8		7	7185.4	
12	7	9559.2		19	5748.5		8	4759.1		7	6659.1	
13	6	10262.1		13	6032.7		4	5744.3		4	6406.1	
14	2	11583.3		12	5818.7		3	5199.4		4	6493.6	
15	1	11547.7		3	6453.4		1	6041.1		4	5977.2	
16	1	8119.1		2	6870.3		1	3286.3		1	4685.0	

as secured by other workers. The percentages given by Gowen and Ragsdale, as referred to in the footnote on the table, represent yearly butterfat records of official testing. Those of Pearl and Miner are from the Scottish Ayrshire records, and represent yearly equivalents of butter-fat. The breed association advanced register requirements were used for calculating the percentages given in the table as coming from this source. The butterfat requirements were the basis of calculation.

Relation of age to milk production expressed in percentage

Comparison made between several sources

BREED	2 YEARS	3 YEARS	4 YEARS	5 YEARS	6 YEARS	7 YEARS	8 YEARS	9 YEARS	10 YEARS	11 YEARS	SOURCE OF DATA
Holstein	66.2	73.7	82.8	90.0	95.3	98.6	100.0	95.5	97.0		College herds*
	68.2	77.6	84.1	94.5	96.3	99.9	100.0				Gowen†
	73.0	83.9	87.1	90.6	96.1	96.6	100.0	95.6	93.5		Ragsdale et al.‡
	66.3	77.5	88.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§ Requirements for Advanced Registry record
Jersey	79.7	85.8	90.9	95.5	98.3	99.3	100.0	99.1	96.6		College Herds*
	67.4	79.2	87.1	92.7	97.2	99.4	100.0				Pearl et al.¶
	73.1	84.4	92.7	95.8	98.5	99.4	100.0	97.3	97.1		Ragsdale et al.‡
	72.6	81.8	90.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§ Requirements for Advanced Registry record
Guernsey	74.2	78.9	84.2	89.1	94.6	98.1	100.0	98.1	95.3		College Herds*
	64.4	80.3	87.1	92.1	95.9	98.3	99.7	100.0			Gowen**
	78.0	85.6	93.2	96.8	98.5	100.0	100.0	99.2	96.9		Ragsdale et al‡
	72.6	81.8	90.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Association§ Requirements for Advanced Registry record
Ayrshire	65.7	69.4	74.8	81.1	87.7	93.5	97.7	100.0	98.9		College Herds*
	70.2	78.7	84.7	88.9	92.3	95.0	97.1	98.6	99.5	100.0	Pearl and Miner††
	74.2	81.4	87.3	92.9	97.5	99.6	100.0	98.1	96.2		Ragsdale et al.‡
	66.3	77.5	88.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Breed Associaton§ Requirements for Advanced Registry record

* These data were secured from experiment station herds. See text, page 547.

† M. S. and J. W. Gowen, Maine Bul. 306, p. 27.

‡ Arthur C. Ragsdale, et al., Jour. of Dairy Science, vii, no. 2, p. 191.

§ Requirements for entry into Advanced Registry as given by breed associations in America. See text, p. 551.

¶ R. Pearl, et al., Maine Bul. 281, p. 92.

** J. W. Gowen, Maine Bul. 311, p. 14–15.

†† R. Pearl and J. R. Miner, Jour. Agr. Res., xvii, p. 285–320.

For the Holstein breed the requirements of the 365-day division were used. The 365-day division of the single letter class of the Jersey register of merit furnished the percentage for this breed. Also the single letter class of the Guernsey requirements was used. For the Ayrshire figure the 365-day fat requirements as adopted in June, 1924, were used.

The use of fat requirements and *fat* production records in comparison with the records of *milk* production of these ages is justified since Gowen (14) has found only slight correlation between changes of age and fat percentage (-0.0546 plus or minus 0.0181). Furthermore, Eckles (15) studied official and non-official records of considerable number and concluded, that "The figures show that the richness of the milk remains practically constant, fluctuating slightly with individuals from year to year, until the animal is past her prime, when it slowly declines with advancing age."

The percentages of table 3 may be used to predict the probable mature production of a young cow when that cow has already made one or more records under non-official test conditions and under conditions that represent good herd management. The calculation involved is division of the record by the percentage of the breed and age represented as given in Table 3. The quotient is multiplied by 100 to get the predicted mature production. Thus a two-year old Ayrshire giving 6570 pounds of milk may be expected to produce $(6570 \div 65.7) \times 100 = 10{,}000$ pounds of milk, when she is mature.

Greater accuracy of prediction will result when there are available two or more records on the cow at immature ages. In such a case each record can be calculated to maturity and the average will represent more nearly, the probable mature production than when only one record is used.

SUMMARY

1. One thousand six hundred eighty-three yearly records from 475 cows are included in a study of the relation between changes in age and milk production of dairy cows. The records were made in eleven experiment station herds in the United States.

2. The Holstein, Jersey, Guernsey and Ayrshire breeds are studied separately.

3. Only records made under other than official test conditions are used.

4. The age of maximum milk production is found to be eight years for the Holstein, Jersey, and Guernsey breeds and for the Ayrshire, nine years, under the conditions studied.

5. The investigation shows that the increase in production is small after six years of age except in the case of Ayrshires which reach maturity one year later than the other three breeds.

6. A table is given comparing the percentages of maturity of milk production at the different ages of this investigation with the percentages as shown by records made under other conditions. The chart included the percentages as derived from the advanced register and register of merit requirements of the several breed associations.

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FURTHER STUDIES ON THE BACTERIAL FLORA OF THE "KINGSTON CHEESE"[1]

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INTRODUCTION

The process of manufacture of "Kingston cheese" was worked out in England by Alec. Todd and Wilfrid Sadler (15) to meet the demand for a cheese of the hard pressed variety, a cheese not too large for the average family and one which could be used with little or no waste. While this cheese is made on the hard pressed principle, the system and procedures employed are such as to give a cheese that will ripen in ten days. The ripe cheese is usually 1 pound in weight, has a flavor of its own and a soft granular texture, rich and buttery. For the past three years this cheese has been manufactured in the laboratories of the University of British Columbia. In a thesis presented by me in April, 1922 (8), detailed references were made to the system on which the cheese is manufactured, and complete records of the cheese made during a large part of the year were presented. The thesis included, moreover, the results of bacteriological examinations of certain of the cheese referred to immediately above. As far as the bacteriological examinations were concerned, the results were very incomplete, yet the technique worked out at that time, and the data obtained, have served as an invaluable preliminary to carrying on the work reported herein.

[1] This paper was presented in partial fulfilment of the requirements for the Degree of Master of Science in Agriculture in the University of British Columbia, May, 1924.

The work was so planned that quantitative bacteriological analyses were to be made of a cheese when one day old, and of a cheese of the same day's make when ready for market, also the organisms occurring with the greatest frequency were to be isolated from the plates of each cheese examined. It has been anticipated that the data thus secured might lead to some information being obtained on the ripening processes of this cheese.

BACTERIOLOGICAL EXAMINATIONS OF THE "KINGSTON CHEESE"

MEDIA EMPLOYED

Beef-peptone-agar, Standard Methods (12).
Glucose-agar 0.5 per cent glucose added to beef-peptone-agar.
Lactose-agar 0.5 per cent lactose added to beef-peptone-agar.
Milk-agar, Ayers and Mudge (1).
MacConkey's neutral-red-bile-salt-lactose-agar (11) for specific reactions of the organisms which ferment lactose to acid and gas.
MacConkey's neutral-red-bile-salt-lactose-broth (11) for detecting the presence of the organisms which ferment lactose to acid and gas.
Nutrient-agar to which specific carbohydrates were added as desired, using brom-cresol-purple as indicator (3) (14).
Nutrient-broth to which specific carbohydrates were added as desired, using brom-thymol-blue as indicator (12).
Peptone-medium for Methyl Red and Voges-Proskauer determinations. Standard Methods (12).
Peptone-medium for Indol Production. Standard Methods (12).
Nitrate-agar. Manual of Methods (14).
Glucose-agar and glucose-gelatin were used throughout for the quantitative plate counts. For comparative purposes lactose-agar and milk-agar (1) were used in addition to the two media mentioned above. The dilution method of quantitative analyses was employed when using litmus-milk and the Voges-Proskauer broth.

Reaction of media

All media, excepting bile-salt media, were adjusted according to hydrogen-ion concentration. The final reaction was pH = 7 using brom-thymol-blue as indicator (12).

Examination and technique

The same technique was used for the bacteriological examinations of the cheese as was worked out during the previous studies on the "Kingston cheese" (8). On arrival at the laboratory the one pound cheese was cut in quarters and the necessary precautions being observed, six samples were taken throughout the cheese, the whole approximating as near as possible 1 gram. The material was placed in a small weighed aluminum dish with a tight fitting cover. The dish was again weighed, and the weight of the cheese determined. The sample was then ground with sand in a sterile glass mortar, and with the aid of a glass rod fitted at one end with a rubber policeman, was washed into a large-mouthed bottle using a known quantity of sterile water. As before (8) an electrically-driven horizontal shaker was used for the mixing of the dilution. The mixing occupied five minutes, and to provide against organisms being carried down by sand, shaking by hand was done for another five minutes. The higher dilutions were then made in water blanks, and it was found that dilutions of 1:500,000 and 1:2,000,000 gave in practice the most satisfactory plates.

Comparative values of certain media

In the work on the "Kingston cheese" reported in 1922 (8) the indications were that glucose agar would give better results than lactose agar. At that time there was not sufficient data on which to form any definite conclusions. During the work herein recorded a comparison was made of glucose agar with lactose agar; and, later of glucose agar with the milk agar of Ayers and Mudge (1). Glucose agar proved to be the most satisfactory medium. Glucose gelatin plates were also made, and as a rule, higher counts were obtained than when using glucose agar. On hot days, however, it was very difficult to keep the gelatin from melting. As is stated on page 556, the dilution method was employed when using litmus milk and the Voges-Proskauer broth respectively. In many instances growth in higher dilutions was observed in the litmus milk than was the case with the Voges-Proskauer medium.

QUANTITATIVE BACTERIOLOGICAL ANALYSES

All quantitative examinations were made in triplicate and the results given below and in table 1 are averages of these three counts. The gelatin plates were incubated for five days at room temperature. The bile-salt broth tubes and bile-salt-agar plates were incubated for forty-eight hours at 37.5°C. All other media were incubated for five days at 22°C. In order to form some idea of the percentage of acid-formers present on the plates, litmus and brom-cresol-purple were used as

TABLE 1

DATES ON WHICH CHEESE WERE MADE	COUNTS MADE ON GLUCOSE AGAR NUMBER OF BACTERIA PER GRAM OF CHEESE	
	Green cheese*	Ripe cheese†
December 12, 1921	565,984,000	353,270,000
August 3, 1923	2,175,000,000	18,000,000
August 7, 1923	2,803,000,000	100,000,000
August 10, 1923	131,100,000	62,150,000
October 3, 1923	110,970,000	55,561,000
October 10, 1923	91,274,000	45,396,000
October 11, 1923	12,824,000	61,000,000
October 18, 1923	123,200,000	68,665,000
October 22, 1923	74,851,000	20,521,000
October 29, 1923	154,452,000	29,016,000

* "Green cheese" refers to the cheese not older than two days from time of making.

† "Ripe cheese" refers to the cheese ready for market, eight to twelve days from time of making.

indicators in a number of glucose-agar-plates. As was experienced before (8) in many instances the indicator in the whole of the medium was changed. On plates where the acid-formers could be differentiated, the number in the majority of cases was 100 per cent of the count and the proportion was not below 97 per cent in any examination.

Twenty cheeses were submitted to a quantitative bacterial analysis. On ten different days the bacterial content of a specific cheese of a certain day's "make," was determined, when the cheese was about twenty-four hours old; and another cheese

of the same day's "make" respectively was examined when ripe about ten days later. It was not possible to examine the same cheese when twenty-four hours old and then ten days old, because the size of the cheese, and the method of sampling, defined on page 557 necessitated the cutting up of the entire cheese for the first determinations. It is to be observed, however, that all the cheese made on any one day were from one vat of milk and from one body of curd which remained in the vat up to the filling of the moulds prior to pressing. Harrison and Connell in their work on cheddar cheese (5) state that

> A source of error in the quantitative bacteriological analysis of cheese is the fact, ... that plugs from different parts of the same cheese, of the same age, vary as much as 30 per cent, in the bacterial content. Further, even in the same plug, portions of equal weight sometimes show as high as 20 per cent of difference in the number of bacteria contained in it.

As yet no attempt has been made to determine if there were any great variations in numbers of bacteria between two cheeses of the same day's "make," but it is to be expected, considering that they were from the same curd, that no greater variation would be found than the 30 per cent found in different parts of a cheddar cheese by Harrison and Connell. In every case, excepting the cheese made on October the eleventh, a considerable reduction was shown in the bacterial count of the ripe cheese when compared with the count of the green cheese of the same day's "make." The number of organisms per gram varied considerably in the different cheeses examined. One cheese a day old gave a count as high as 4,000,000,000 microörganisms per gram; and another, also a day old, had a count as low as 9,000,000 microörganisms per gram. The counts in the ripe cheese varied from 147,000,000 to 12,000,000 per gram. Though there was this great difference in the number of organisms found in the different cheeses, there was no great difference in the quality of the cheese as judged for market. More detailed results will be found in table 1.

As in the examinations of two years ago there has been no attempt to make anaerobic examinations of the cheese. It is fully realized that this part of the work should be undertaken; but it was not possible simultaneously to do both aerobic and anaerobic examinations, and it was decided for the time being to concentrate on work under aerobic conditions. It is to be desired that an inquiry into the anaerobic flora of the cheese shall be instituted.

An effort was made to conduct histological examinations of the cheese in order to get some idea of the grouping of the organisms; and to secure counts of the bacteria by the microscopic method as recommended by Hucker (6). Thus far, time has not permitted the doing of other than preliminary work on this phase of the investigation. At a later date it is to be desired that the examination of histological specimens shall also receive consideration.

QUALITATIVE BACTERIOLOGICAL ANALYSES

Colonies which appeared to occur with the greatest frequency were taken off the plates and retained in pure culture. A large percentage of these colonies were similar to the small sub-surface and surface colonies—group II—reported in the studies undertaken in 1922 (8). Great difficulty was found at that time (8) in keeping the organisms of group II alive long enough to do even a preliminary examination of them. Also, the growth on agar slants was found to be insufficient for inoculation purposes. In order to avoid the difficulties mentioned above, the following procedure was evolved and followed with good results throughout the present investigation: The colonies were fished off gelatin plates in the manner found so advantageous in the later stages of the work reported before (8); a small lump of gelatin containing the colony was lifted out of the plate, put into Voges-Proskauer broth and held at 37.5°C. till the gelatin melted, freeing the colony. The tubes of broth were then incubated at 22°C. When a cloudiness in the broth indicated that growth had taken place, this broth was used to inoculate the desired culture media. A small quantity of the broth was drawn up into a sterile pipette

and all tubes of culture media inoculated with one drop each from the point of the pipette. The glucose broth of the Voges-Proskauer test was used because it was the only liquid medium in which the organisms of group II (8) could be grown satisfactorily.

As a preliminary examination, all cultures were stained by Gram and the reactions to litmus-milk, glucose, lactose and sucrose were recorded. Later it seemed desirable to determine the reaction to maltose, glycerol, salicin and mannitol. The results of the above examinations are recorded in table 2. The agar slants recommended by Conn and Hucker (3) and given in the "Manual of Methods for Pure Culture Study of Bacteria" (14) were used for the determination of acid and gas in all the carbohydrate media employed.

It has been noted above, that, for these determinations, one drop, from a finely-bored pipette, of the Voges-Proskauer glucose solution was used for the inoculation in each case. The possibility of the infinitesimal amount of glucose present in the drop of culture being sufficient to show acidity in the media other than glucose suggested itself. To check this, ten tubes of nutrient agar slants containing indicator, but no sugar, were inoculated at the same time as the other media. The control tubes remained neutral. Ten tubes of nutrient-agar were not considered as sufficient check, but the fact that a large percentage of the cultures did not produce acid on lactose-agar was considered as conclusive proof that when the minute quantity of glucose present in the drop of inoculating material was fermented there was not sufficient acid produced to change the color of the indicator in the media inoculated.

One hundred seventeen organisms were isolated from plates made of the cheese, and 5 organisms from plates made of the starter used in the making of the cheese. When the reactions of the cultures to maltose, salicin, glycerol and mannitol were determined, it was found that 23 organisms had died. The information already obtained about them, however, was enough to show to which main group they belonged. The 117 organisms divide themselves into five main groups.

TABLE 2

Morphology, cultural features and physiological reactions of organisms isolated from "Kingston cheese"

Culture number	Number of cultures represented	Morphology	Gram stain	Motility	Chromogenesis Winslow's chart	Gelatin liquefied	Glucose	Lactose	Sucrose	Glycerol	Salicin	Mannitol	Maltose	Levulose	Raffinose	Gelactose	Arabinose	Trehalose	Dextrin	Inulin	Dulcitol	Indol	Methyl red	Voges-Proskauer	Nitrates reduced	Milk: Acid	Milk: Gas	Milk: Clot	Milk: Peptonized
115	6	Cocci 0.75–1 μ in diameter	+			−	+−	+−	−−																	+	−	+	−
121	16	Cocci 1–1.5×0.75–1 μ	+			−	+−	+−	−−																	+	−	+	−
212	1	Cocci 1–1.5×0.75–1 μ	+			−	+−	+−	−−	−−	−−	−−	−−													+	−	+	−
136	14	Cocci 0.75–1 μ in diameter	+			−	+−	+−	−−	−−	−−	−−	+−													+	−	+	−
135	32	Cocci 1–1.5×0.75–1 μ	+			−	+−	+−	−−	−−	−−	−−	+−													+	−	+	−
172	3	Cocci 1–1.5×0.75–1 μ	+			−	+−	+−	−−	−−	+−	−−	+−													+	−	+	−
156	5	Cocci 0.75–1 μ in diameter	+			−	+−	+−	+−	−−	+−	+−	+−													+	−	+	−
152	2	Cocci 1–1.5×0.75–1 μ	+			−	+−	+−	+−	−−	+−	+−	+−													+	−	+	−
101	3	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	+−	+−	+−	−−		+−											+	−	+	−
102	1	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	−−	+−	+−	+−	+−	+−	+−	+−	+−	+−	+−						+	−	+	−
103	2	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	+−	+−	−−	−−		+−											+	−	+	−
104	14	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	+−	+−	+−	+−		+−		+−	+−	+−							+	−	+	−
M12	2	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	−−	+−	+−	+−		+−		+−	+−	−−							+	−	+	−
M9	1	Rods 1 μ×1–10 μ	+			−	+−	+−	+−	−−	+−	+−	−−		+−											+	−	+	−
119	5	Short rods	−	−		−	++	++	+−	+−	+−	+−	+−								+−	−	−	−	+	+	−	+	−
124	3	Short rods	−	−		−	++	++	−−	++	++	++	++								−−	+	+	−	+	+	−	+	−
143	5	Short rods	−	+		−	++	++	−−	++	++	++	++								++	+	+	−	+	+	+	+	−
206	3	Short rods	−	−		−	++	++	++	++	++	++	++								++	−	−	−	+	+	+	+	−
110	1	Spore forming rods	−	+		−	+−	+−	+−	+−	+−	+−	+−													+	−	−	−
133	1	Micrococci	+		Yellow	+	+−	−−	−−	+−	+−	+−	+−													−	−	−	+
214	1	Staphylococci	+		White	+	+−	−−	+−																	−	−	+	−
148	1	Micrococci	+		White	−	+−	+−	+−	−−	−−	+−	+−												+	+	−	+	−

Reactions: + positive; −negative; ++ acid and gas; +−acid; −− no action.

GROUP I

In this group are found organisms isolated from pin-head colonies growing more particularly under the surface of the media, Gram-positive, spherical, occurring in chains, ones and twos and in clumps. Each strain ferments glucose and lactose to acid, produces acid and clot in litmus milk and fails to liquefy gelatin. Seventy-nine strains find themselves in this group. Within the group, however, specific strains vary in the size of the cells and in the action on certain of the carbohydrates other than glucose and lactose. In table 2 the variations in size of cell and in the action on the carbohydrates other than glucose and lactose are recognized and sub-groups are established. Of the 79 strains thus placed in sub-groups, cultures 115, 121, 212, 136, 135, 172, 156 and 152 prove to be representative of the number of strains respectively, as shown in the second column of the plate. Cultures 156 and 152 representing 5 and 2 strains respectively, agree in the essential characteristics with the description of Streptococcus lactis (Lister) according to Bergey's Manual of Determinative Bacteriology (13). Strains represented by Cultures 115, 121, 212, 136, 135 and 172, however, cannot be classified as typical forms of Streptococcus lactis (Lister) because of the failure to act on salicin, on mannitol, or on both (13). It was also noted that only 3 strains coming in this group and isolated from cheese, ferment salicin and mannitol to acid, while three more strains ferment salicin only. It may be that due to having passed through cheese the majority of the organisms which find themselves in group I had lost the power of fermenting salicin and mannitol, 3 strains had lost the power of fermenting mannitol only, while 3 strains had retained the power of fermenting both these carbohydrates. It would seem that with forms of Streptococcus lactis (Lister) attenuation takes place in passing through cheese. Support for this suggestion is found in the work of Lloyd on cheddar cheese (9). From well-matured cheese he isolated strains of true lactic acid producing organisms which he defined as Bacillus acidi lactici—now considered as Streptococcus lactis (Lister) (13) (9)—which failed to clot milk. Further, of the

organisms isolated from cheese 3 only of the strains included in this group ferment salicin and mannitol to acid, while three of the strains ferment salicin to acid but fail to act on mannitol. The remainder of those cultures isolated from cheese and included in this group have no action on either salicin or mannitol. Of the organisms comprising group I, every strain is found to be identical with one or more of the strains isolated by Hucker from cheddar cheese (7). Each of his strains is classified as Streptococcus lactis (Lister) (7). In studying the organisms placed here in group I, the reactions to salicin, mannitol and maltose, have been determined in addition to the reactions recorded by Hucker for his strains. Consequently, while Hucker (7) classified his organisms as Streptococcus lactis (Lister) the additional reactions recorded for the cultures—group I—of this report permit of a more specific classification, following Bergey (13). Accordingly, cultures 152 and 156 are placed as typical forms of Streptococcus lactis (Lister) (13), after Bergey; and Cultures 115, 121, 212, 136, 135 and 172, on account of their failure to ferment mannitol or salicin, or mannitol and salicinin, are placed as attenuated forms of Streptococcus lactis (Lister) (13).

GROUP II (GRAM-POSITIVE ROD-SHAPED ACID FORMERS)

Nineteen cultures represented in table 2 by cultures 101, 102, 103, 104, M12 and M9 are Gram-positive non-spore bearing rods with round ends, varying in length from 1 to 10μ, the majority approximating the minimum length rather than the maximum. There was a tendency to form short chains in the broth as used for the Voges-Proskauer test and to a somewhat lesser extent on agar. Good growth took place both at 22° and 40°C. All the cultures, with the exception of cultures 204 and 205 were picked off agar and gelatin plates incubated at 22°C. for five days, while cultures 204 and 205 were taken off plates incubated at 40°C. The strains do not liquefy gelatin but form a clean acid clot in litmus-milk in from fourteen to eighteen days at 40°C. followed by bleaching. Gas is not formed in any of the carbohydrates and glucose, lactose, sucrose, salicin and raffinose are fermented to acid. These cultures of group II seem

to bear a marked resemblance to Orla-Jensen's Streptobacterium (10) in that they are Gram-positive rods tending to form chains, growing at 22° to 40°C. and fermenting salicin to acid. Smears made from nutrient agar slants showed a large number of short rods which might be mistaken almost for diplococci with a number of rods about 10μ long. These smears were almost identical in appearance with Orla-Jensen's Streptobacterium plantarium number 18, agar streak one day at 30° plate XLII (10). According to Bergey's Manual (13) the cultural and morphological characteristics of the organisms here under discussion would place them in the genus Lactobacillus (Beijerinck) (13). It was impossible to isolate organisms of this type from the freshly made cheese though several attempts were made. All the cultures mentioned above were from cheese eight to ten days old. It has been known that Lactobacilli are found in mature cheddar cheese and it is interesting to know, that in a cheese which ripens so quickly, Lactobacilli are present in quite considerable numbers.

Cultures 101 and M9 do not ferment maltose, but ferment mannitol to acid. The cultural and morphological characteristics of these two organisms would seem to warrant the classifying of them as of the Lactobacillus bulgaricus (Grigoroff) type (13) and as of Rahe's type "D" (13).

Cultures 102 and 104 ferment maltose, mannitol, raffinose, dextrin, arabinose and trehalose to acid, trehalose being fermented slowly. The cultural characteristics of these organisms, and particularly their action on the carbohydrates, would require that they be classified as Lactobacillus cucumeris (Henneberg) (13). However, the length of the cells of cultures 102 and 104 vary from 1 to 10μ while the length of the cells recorded for Lactobacillus cucumeris are from 1.5 to 2μ. With the exception of the reaction to trehalose, the morphological and cultural characteristics of cultures 102 and 104 appear to be identical with those recorded for Lactobacillus plantari (Orla-Jensen) (13). Further these two organisms and Lactobacillus plantari, clot milk, while Lactobacillus cucumeris does not. The action of Lactobacillus cucumeris on dextrin is much slower as compared with its reaction to trehalose, while with strains 102

and 104, the reverse is the case. Trehalose is the sugar which, according to Bergey (13) divides Lactobacillus cucumeris and Lactobacillus plantari. It is obvious, therefore, considering the characteristics of Lactobacillus cucumeris and Lactobacillus plantari respectively, that cultures 102 and 104 are to be placed with one or other of these two known strains according to the relative importance which is to be attached to the fermentation of trehalose on the one hand, and to the size of cells and the clotting of milk on the other hand. On the whole following Bergey, the evidence submitted appears to be in favour of Lactobacillus cucumeris; and cultures 102 and 104 are classified as types of Lactobacillus cucumeris (Henneberg) after Bergey (13).

Culture 103 does not ferment maltose and mannitol. The action on the carbohydrates would place culture 103 as either Lactobacillus caucasicus (Kern) (13) or Lactobacillus boas-oppleri (Boas and Oppler) (13). As neither Lactobacillus caucasicus (13) nor Lactobacillus boas-oppleri will grow on gelatin at 22°C. and as culture 103 was isolated from a gelatin plate incubated at room temperature, it cannot be classified either as Lactobacillus caucasicus or Lactobacillus boas-oppleri, but is placed within the type species Lactobacillus caucasicus (Kern) (13).

Culture M12 ferments maltose, mannitol and raffinose to acid. The ability of this organism to ferment raffinose without fermenting dextrin does not permit of it being classified by Bergey's Determinative Bacteriology (13) other than as being a member of the genus Lactobacillus and coming within the type species Lactobacillus caucasicus (Kern) (13).

GROUP III (GRAM-NEGATIVE NON-SPORE FORMING RODS, WHICH FERMENT LACTOSE TO ACID AND GAS)

There are 16 organisms in this group represented on table 2 by cultures 119, 124, 127 and 206. All were short Gram-negative rods which ferment glucose and lactose to acid and gas, clot milk, reduce nitrates to nitrites, but fail to liquefy gelatin. The characteristics noted above, and the negative reaction to the Voges-Proskauer test would place these strains in the genus Escherichia (13).

Of the organisms in this group, culture 119 is a non-motile rod which ferments sucrose, salicin, maltose, glycerol, dulcitol and mannitol to acid; produces sliminess on agar, in peptone broth and in milk, but fails to produce either indol or acetyl-methyl-carbinol. When first isolated this strain fermented sucrose to acid and gas, but after being kept on artificial media for some months lost the faculty of producing gas on this carbohydrate. In its ability to produce sliminess on certain media, to ferment glucose and lactose to acid and gas and its inability to produce indol, culture 119 shows a resemblance to Bacterium aerogenes (Escherich) as described by Buchanan and Hammer (2). Their Bacterium aerogenes is shown as fermenting carbohydrates, other than glucose and lactose, to acid and gas, while this strain ferments them to acid only. Bacterium aerogenes (Escherich) becomes Aerobacter aerogenes (Escherich) in Bergey's Determinative Bacteriology (13). Following Bergey (13) culture 119 cannot be classified as Aerobacter aerogenes, for it fails to produce acetyl-methyl-carbinol, but based on the sum of the characteristics recorded the strain finds itself within the genus Escherichia. Of the species in this genus included in Determinative Bacteriology (13) it appears that Escherichia astheniae presents the features with which the culture under discussion most closely aligns itself. This culture, therefore, is here considered as be'ng an atypical form of Escherichia astheniae (Dawson) (13).

Culture 124 is a non-motile rod, ferments glucose and lactose to acid and gas, but fails to act on sucrose and salicin. But for the fact that culture 124 is non-motile, it could be classified as Escherichia paragrunthali (Castellani and Chalmers) (13). This lack of motility, however, prohibits it being classified as a true form of Escherichia paragrunthali and it is classified as of the type Escherichia paragrunthali (Castellani and Chalmers) (13).

Culture 143 is a strain of motile rods which forms indol, ferments salicin and dulcitol to acid and gas, but fails to ferment sucrose. The ability of this strain to reduce nitrates to nitrites, to ferment glucose, lactose, salicin and dulcitol to acid and gas, the inability to produce acid in sucrose and to form acetyl-

methyl-carbinol places this organism as a true type of Escherichia coli (Escherich) Castellani and Chalmers (13).

Culture 206 is a non-motile rod which ferments salicin and sucrose to acid and gas, but does not produce indol or give the Voges-Proskauer reaction. The ability of the culture to ferment sucrose and lactose to acid and gas, the inability to produce indol or give the Voges-Proskauer reaction, and its failure to show motility would suggest that it be placed as Escherichia astheniae (Dawson) (13). Escherichia astheniae, however, has no action on salicin, while culture 206, as noted above, ferments this carbohydrate to acid and gas. This difference in action on salicin would not permit the classifying of this strain as a typical form of Escherichia astheniae and it is classified here as an atypical form of Eschrichia astheniae (Dawson) (13).

GROUP IV (SPORE-BEARING RODS)

Only one spore-bearing organism, culture 110, was found in this investigation. The strain is a Gram-negative motile rod 2 to 4μ in length, the cells occurring singly and in pairs. The spores are terminal and the rods are swollen at sporulation giving the cell the appearance of a tadpole. The growth on agar is pale yellowish white after forty-eight hours, gelatin is not liquefied and acid is formed in milk but no clot is produced. Glucose, lactose, and sucrose are fermented to acid. The cultural and morphological characteristics of culture 110 appear to be identical with those recorded for Bacillus pseudotetanicus (Kruse) (13) with the exception of the action on the carbohydrates; Bacillus pseudotetanicus showing no action on the carbohydrates. Culture 110 bears a marked resemblance also to Bacillus circulans (Jordan) (13) but the 2 cultures do not agree in the reaction to the Gram stain. It is suggested that in spite of the difference noted with respect to the action to the Gram stain, culture 110 shall be considered as being of the type Bacillus circulans (Jordan) (13).

GROUP V (COCCUS FORMS)

Culture 133 is a minute Gram-positive coccus about 0.5μ in diameter, which liquefies gelatin, ferments glucose, maltose,

salicin and mannitol to acid, but has no action on lactose or sucrose. This strain bears a marked resemblance to Micrococcus lactis varians of Conn, Esten and Stocking (4). These investigators found that some forms of Micrococcus lactis varians did not ferment lactose and sucrose, characteristics applying equally to culture 133. Comparing the characteristics as a whole, the strain agrees even more closely with Micrococcus lactis varians (type A) of Conn, Esten and Stocking (4) both as to the formation of acid in glucose, and in the curdling and digesting of milk without the formation of acid. The resemblance, however, of the strain to Micrococcus varians (Dyar) Conn (13) is not so pronounced; yet Micrococcus varians (Dyar) Conn is considered in Bergey's Determinative Bacteriology as being synonymous with Micrococcus lactis varians of Conn, Esten and Stocking. Hence, it would seem that culture 133 is to be classified as of the type Micrococcus varians (Dyar) Conn (13).

Culture 148 is a strain of Gram-positive cocci growing in clumps. It fails to liquefy gelatin, forms a clean acid clot in milk, reduces nitrates to nitrites and ferments glucose, lactose and sucrose to acid. No action on maltose, salicin or inulin has been noted. The growth is good on artificial media and the pigment on agar after fourteen days, according to the Winslows' chart (16), is Light Cadmium Yellow, Chrom III, designated by these workers as "white." The characteristics recorded would suggest that the strain be placed in the genus Staphylococcus (13). To take the classification to a more specific stage, the failure of the organism to liquefy gelatin and the ability with which lactose is fermented indicate a close resemblance of the strain to Staphylococcus tetragenus (Koch-Gaffky) (13). The identity of the 2 strains cannot be accepted without qualification for culture 148 reduces nitrates to nitrites, and under the microscope appears as cells in clumps, while Staphylococcus tetragenus (13) does not reduce nitrates and the cells appear in groups of four. Winslow and Winslow (16) state that Albococcus tetragenus (Gaffky)—albococcus being a genus since absorbed in the genus Staphylococcus—is closely related to Albococcus candidus

(Cohn) and in describing Albococcus candidus, Winslow et al. (17) make the following statement

> The second type in abundance in our study, and the type found most commonly on the skin after St. epidermidis by Gordon, is the form which ferments lactose but fails to liquefy gelatin, identified by Winslows as Albococcus candidus and now to be called Staphylococcus candidus. Our strain, however, reduced nitrates and generally clotted milk which Gordon's type did not. Three strains sent to the museum collection as Micrococcus tetragenus all belonged to this group. None of them reduced nitrates and results are variable in milk and in regard to ammonia production.

The strain recorded here agrees with the above description of Staphylococcus candidus with respect to the clotting of milk and the reduction of nitrates to nitrites. In table 10 of the same report (17) Winslow et al. show 20 of their strains as fermenting maltose. Culture 148 fails to ferment this sugar but in all other respects it appears to be identical with their cultures as cited. The organism originally described by Cohn was placed by him in the genus Micrococcus as Micrococcus candidus (16). Later organisms showing similar characteristics to Cohn's Micrococcus were placed by the Winslows (16) within the type center Albococcus candidus (Cohn) and later as Staphylococcus candidus (Cohn) (17). The Committee of the Society of American Bacteriologists in Bergey's Determinative Bacteriology (13) have again placed organisms of this type in the genus Micrococcus, as Micrococcus candidus (Cohn) (13). Though culture 148 differs from Micrococcus candidus (Cohn) (13) in the action to nitrates, in the main the characteristics are identical and culture 148 is classified here as of the type Micrococcus candidus (Cohn) (13).

Culture 214 is a Gram-positive coccus, occurring in irregular groups. The strain liquefies gelatin, stratiform, produces a white pigment on agar, ferments glucose and sucrose to acid, but no action on lactose can be noted. This culture died before it could be investigated further. According to the cultural and morphological features determined, however, culture 214 would be placed in the genus Staphylococcus, and of the type species

Staphylococcus aureus (Rosenbach) (13). The inability of the strain to ferment lactose, does not permit of it being classified more specifically. Winslow, Rothberg and Parsons in the White and Orange Staphylococci (17) show 10 cultures in their table 8, which are white pigment formers, liquefying gelatin, but failing to ferment lactose. Later the same workers say (17): "The lactose-negative gelatin-positive type of white pigment producers appears in our study, as in that of Gordon, to be a rarer one, and this form, as well as the forms which exhibit miscellaneous fermentative reactions may best be left for the present without specific names." It is felt that culture 214 should be included in this group. Culture 214 is, therefore, left for the present as of the type species Staphylococcus aureus (Rosenbach) (13).

OBSERVATIONS

The technique evolved when engaged on the work in 1922 (8) has been adopted throughout the present investigation with pronounced satisfaction. Further, the present paper confirms the previous findings (8) that for the determinations of the bacterial flora of "Kingston cheese" glucose-agar and glucose-gelatin are the most satisfactory media.

The total number of organisms present in the "Kingston cheese" both in the cheese when newly made and the cheese when ten days old, is found to approximate very closely the numbers recorded in the work on cheddar cheese. Though the total number of organisms present in the "Kingston cheese" ten days old is not constant, and may vary, as this investigation shows from 20,000,000 to 350,000,000 bacteria per gram, the cheese examined in each case were normal both as to texture and flavor.

Though no definite effort was made to determine the percentage of acid formers, the results obtained showed that at least 97 per cent of the flora were acid formers.

This paper would seem to indicate that the organisms which occur with the greatest frequency in the cheese when one day old are those of the Streptococcus lactis (Lister) type, and in cheese of the same day's "make" when ten days old are those

of the Streptococcus lactis (Lister) type and those of the Lactobacillus (Beijerinck) type. It appears to be well established that the successful ripening of cheddar cheese is very largely dependent upon the bacterial flora determined according to the recorded literature. The work herein presented, quite definitely shows that the flora of the "Kingston cheese" is very similar, if not identical with the flora recorded for cheddar cheese. Yet in the case of the "Kingston cheese" we have a cheese which is mature in ten days after making, while a cheddar cheese requires from three to six months to arrive at maturity.

It would seem, therefore, that in contemplating the factors determining the successful ripening or maturing of the "Kingston cheese" due regard must be paid to the system of manufacture adopted, to the temperature at which ripening takes place and to all the processes associated with the management of the cheese, for as far as this paper can define, the bacterial flora of the "Kingston cheese" is clearly identical with that of the cheddar cheese.

SUMMARY

A brief résumé is given of the system adopted in the making of the "Kingston cheese" and the specific characteristics of the cheese are noted.

Bacteriological analyses have been made of twenty cheeses manufactured on ten different days, ten cheeses when one day old and ten cheeses of the same day's "make" when mature at ten days after making. The results of the analyses made of these twenty cheeses are recorded in table 1.

A list of the media employed is recorded and a description of the method of examination of the cheese is given. Several varieties of media were used with the object of determining which were best adapted for the determination of the bacterial analyses of the "Kingston cheese," glucose-agar and glucose-gelatin proving to be most satisfactory.

One hundred seventeen organisms have been isolated from plates made of the cheese and 5 from plates made of the starter used in the making of the cheese. As far as possible the organ-

isms were those which appeared to occur with the greatest degree of frequency. Though 18 of the 122 organisms isolated were of the genus Escherichia this does not necessarily represent the percentage of organisms of that Genus present in the cheese. The strains were taken from MacConkey's broth tubes and MacConkey's agar plates in order to determine the species of gas formers occurring with the greatest frequency.

The morphology, cultural features and physiological reactions of organisms isolated from "Kingston cheese" are given in table 2.

The 122 organisms isolated are placed in five main groups, and are classified according to Bergey's Determinative Bacteriology (13).

Group I—Streptococcus lactis (Lister) types

Seventy-nine of the 117 cultures recorded in this paper find themselves in this group, and are classified as follows.

Cultures 156 and 152 representing in all 7 strains are classified as Streptococcus lactis (Lister) (13).

Cultures 115, 121, 212, 136, 135, and 172, representing 72 strains are placed as attenuated forms of Streptococcus lactis (Lister) (13).

Group II—Lactobacillus (Beijerinck) types (13)

Twenty-three strains are placed in this main group and are classified as to species.

Cultures 101 and M9 representing 4 cultures are classified as Lactobacillus bulgaricus (Grigoroff) (13) and of the type "D" (Rahe) (13).

Cultures 102 and 104 representing 15 strains are placed as atypical forms of Lactobacillus cucumeris (Henneberg) (13).

Cultures 103 and M12 representing 2 strains each are classified as of the type species Lactobacillus caucasicus (Kern) (13).

Group III—Gram-negative, lactose, fermenting rods

In all there are 16 strains in this group represented by 4 cultures.

Eight strains represented by cultures 119 and 206 are classified as atypical forms of Escherichia astheniae (Dawson) (13).

Three strains represented by culture 124 are considered as atypical forms of Escherichia paragrunthali (Castellani and Chalmers) (13).

Five strains represented by culture 143 are classified as Escherichia coli (Escherich) Castellani and Chalmers (13).

Group IV—Spore-bearing rods

Only 1 culture of spore-forming rods was found in this investigation.

Culture 110 is placed as an atypical form of Bacillus circulans (Jordan) (13).

Group V—Coccus forms other than Streptococcus lactis (Lister)

Three cultures find themselves in this group, 2 of which are Micrococci and 1 a Staphylococcus.

One strain, culture 133, is classified as of the type Micrococcus varians (Dyar) Conn (13).

One strain, culture 214, is placed as of the type species Staphylococcus aureus (Rosenbach) (13).

One strain culture 148, is considered to be within the type species Micrococcus candidus (Cohn) (13).

Observations on the data presented are offered.

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A COMPARATIVE STUDY OF METHODS FOR DETERMINING TOTAL SOLIDS IN ICE CREAM

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In this paper are presented a second series of results involving the comparative study of methods for determining total solids in dairy products. The first report on this study was made by Fisher and Rice (1) and dealt with the determination of total solids in sweetened condensed milk. As reported, twenty samples of condensed milk were tested for total solids by the official, the Mojonnier and a modified method suggested by the authors. Considering the properties of sweetened condensed milk, the three methods gave reasonably close results. The Mojonnier method averaged 0.57 per cent and the modified 0.47 per cent higher than the official. In view of these results and the greatly felt need for a simple, economical, but accurate total solids test for ice cream, the second series of trials were made on this dairy product.

EXPERIMENTAL WORK

Fifty samples of ice cream were analyzed by both the Mojonnier and a modified test similar to the one suggested for sweetened condensed milk (1). Since there is no recognized official test available, the authors adapted a total solids test from the official method for sweetened condensed milk. In the following pages this method is referred to as "adapted official." Twelve of the samples of ice cream were tested for total solids by the adapted official and compared with the Mojonnier and the modified method.

The procedure of each was as follows.

1. Adapted test from official method for condensed milk

One-half pint samples of ice cream were gradually warmed by placing in a hot water bath, the temperature of which was not over 30°C. Samples were allowed to remain in the water bath until they reached about 25°C. and were thoroughly melted. Exactly 20 grams of thoroughly mixed sample were weighed into a beaker, and transferred to a 100 cc. volumetric flask with the aid of hot redistilled water. This was cooled and made up to the mark with redistilled water. Exactly 10 cc. of the solution was pipetted into a previously dried and tared aluminum dish and the free moisture evaporated off over water bath. This required about twenty minutes. Dishes were then transferred to a water oven and drying continued at the temperature of boiling water until constant weight was reached.

The dishes used in this test were ordinary flat-bottomed aluminum dishes about 5 cm. in diameter. Empty dishes were dried in the water oven and then cooled for a similar period in an ordinary calcium chloride desiccator; they were always kept covered while weighing. The percentage of solids were calculated from the final weight of solids in the dish.

2. Modified method (as adapted from modified method for sweetened condensed milk)

The sample was prepared in a similar manner as that outlined above by melting at about 25°C. in water bath. Approximately 1 gram of well mixed sample was weighed directly into a previously dried and tared aluminum dish. One cubic centimeter of hot redistilled water was added and thoroughly mixed. The procedure from this step on was similar to the modified test for sweetened condensed milk and was briefly as follows:

After adding the distilled water, the dishes were placed on an electric hot plate, kept at 180°C. and the moisture carefully evaporated off until first traces of brown appeared. Drying was then continued in a water oven at the temperature of boiling water until constant weight was reached.

The dishes used in this modified test, as in the adapted official test, were ordinary flat-bottomed aluminum dishes about 5 centimeters in diameter. Empty dishes were dried for ten minutes in water oven and then cooled for a similar period in an ordinary calcium chloride desiccator. They were always kept covered while weighing.

3. The Mojonnier method

The procedure for determining total solids in ice cream as described in Technical Control of Dairy Products by Mojonnier and Troy, page 126, was followed exactly.

EXPERIMENTAL RESULTS

The percentage of total solids obtained by each of the three methods for twelve samples of ice cream are reported in table 1. A study of the table shows that in most cases the results obtained by the three methods compare fairly closely. In all but the first two samples the Mojonnier method gave the highest results, the adapted official the lowest, while the modified method usually gave results between the adapted official and the Mojonnier. It is interesting to recall that the same observations were made in regard to the results obtained by the three methods in the case of sweetened condensed milk.

Table 2 shows the variation in the modified and Mojonnier methods from the adapted official. On 12 samples the Mojonnier test averaged 0.44 per cent higher than the adapted official while the modified test averaged 0.23 per cent higher. Fifty-eight per cent of the results by the modified test checked within 0.25 per cent with the adapted official while all checked within 0.5 per cent. In the case of the Mojonnier 16.6 per cent checked within 0.25 and 50 per cent checked within 0.5 per cent.

If one takes the adapted official as the standard for comparison, the modified test gave more favorable results than the Mojonnier. Furthermore as already pointed out in the first paper (1), the modified method is simpler and more rapid than the official. The equipment needed is inexpensive and frequently on hand in the laboratory.

TABLE 1

Percentage of solids in samples of ice cream according to three methods studied

SAMPLE NUMBER	ADAPTED TEST FROM OFFICIAL METHOD FOR SWEETENED CONDENSED MILK	MODIFIED METHOD	MOJONNIER
1a	36.71	36.51	36.72
1b	36.64	36.58	36.68
2a	38.01	38.27	38.51
2b	38.10	38.17	38.55
3a	38.05	38.21	38.31
3b	38.04	38.19	38.33
4a	40.39	40.59	40.86
4b	40.35	40.48	40.88
5a	40.07	40.41	40.62
5b	40.04	40.47	40.64
6a	38.03	38.37	38.80
6b	38.05	38.42	38.92

TABLE 2

Variation percentage of solids in ice cream as obtained by the three methods studied

SAMPLE NUMBER	MODIFIED FROM ADAPTED OFFICIAL	MOJONNIER FROM ADAPTED OFFICIAL
1a	−0.20	+0.01
1b	−0.06	+0.04
2a	+0.26	+0.54
2b	+0.07	+0.45
3a	+0.16	+0.26
3b	+0.15	+0.29
4a	+0.20	+0.47
4b	+0.13	+0.53
5a	+0.34	+0.55
5b	+0.43	+0.60
6a	+0.34	+0.77
6b	+0.37	+0.87
Average variation........	0.226	0.483
	58 per cent within 0.25 per cent of adapted official 100 per cent within 0.50 per cent of adapted official method	16.6 per cent within 0.25 per cent of adapted official 50 per cent within 0.50 per cent of adapted official method

The modified test therefore would recommend itself as a simple, economic and accurate total solids test for use in ice cream factories and laboratories not provided with the equipment required for the Mojonnier method.

Realizing that results on twelve samples are not sufficient data on which to draw definite conclusions thirty-eight additional samples of ice cream were analyzed for total solids by the modified and Mojonnier method. We were desirous also of analyzing these samples by the adapted official, but this test while undoubtedly accurate and reliable is laborious and time consuming and therefore not well suited for factory work.

As the modified method compared closely with the official in both the 20 samples of condensed milk (1) and the 12 samples of ice cream it was decided to compare only the modified and the Mojonnier method in the next series of trials.

The percentages of total solids obtained by these two methods on 50 samples of ice cream are given in table 3. The results on these 50 tests confirm previous observations in that in all cases the modified method gave slightly lower results than the Mojonnier method. However, as brought out in the following summary, the two methods checked reasonably closely:

34 samples or 68 per cent checked within 0.25 per cent
40 samples or 80 per cent checked within 0.30 per cent
47 samples or 94 per cent checked within 0.40 per cent
48 samples or 96 per cent checked within 0.50 per cent

The average variation as pointed out at the bottom of table 3, was 0.223 per cent. This means that on 50 samples the modified test averaged 0.223 per cent lower than the Mojonnier method. When it is recalled that the modified averaged 0.226 per cent higher than the adapted official method, this fact becomes significant. It means that the modified method gives results about half way between the adapted official and the Mojonnier method.

In view of these facts, it seemed desirable to further standardize the procedure of the modified method, by establishing the average time required for the ice cream to reach constant weight.

at the end of two hours drying in the water oven. Of thé other 8 samples 7 lost less than 0.20 per cent from the time they had been dried for two hours until they reached constant weight.

Since for practical factory purposes, tests for total solids are usually satisfactory when they check within 0.25 of one per cent

TABLE 4

Time required for solids in ice cream to reach constant weight in modified test

SAMPLE NUMBER	PER CENT OF TOTAL SOLIDS AFTER DRYING FOR				PER CENT MOISTURE LOST IN VARIOUS PERIODS OF DRYING IN WATER OVEN		
	2 hours	2½ hours	3 hours	3½ hours	2 to 2½ hours	2½ to 3 hours	3 to 3½ hours
1	37.79	37.76	37.65	37.65	0.03	0.11	0
2	35.11	35.07	34.99	34.99	0.04	0.08	0
3	36.90	36.88	36.81	36.81	0.02	0.07	0
4	37.54	37.54	37.42	37.42	0	0.12	0
5	38.75	38.75	38.62	38.62	0	0.13	0
6	38.57	38.50	38.32	38.32	0.07	0.18	0
7	38.66	38.63	38.54	38.54	0.03	0.09	0
8	37.14	37.14	37.14	37.14	0	0	0
9	38.25	38.21	38.14	38.14	0.04	0.07	0
10	39.60	39.60	39.60	39.60	0	0	0
11	42.41	42.41	42.41	42.41	0	0	0
12	37.00	37.00	37.00	37.00	0	0	0
13	35.56	35.56	35.56	35.56	0	0	0
14	38.32	38.27	38.27	38.27	0.05	0	0
15	40.45	40.41	40.41	40.41	0.04	0	0
16	38.37	38.37	38.37	38.37	0	0	0
17	37.96	37.87	37.87	37.87	0.09	0	0
18	40.63	40.58	40.58	40.57	0.05	0	0.01
19	40.50	40.50	40.48	40.48	0	0.02	0
20	40.69	40.69	40.69	40.69	0	0	0
21	40.60	40.60	40.57	40.57	0	0.03	0
22	40.43	40.43	40.43	40.43	0	0	0
23	38.66	38.62	38.62	38.62	0.04	0	0
Average loss of moisture....................					0.021	0.041	0

and since in commercial work the time required to make the test is frequently more important than absolute accuracy, it seems justifiable to consider two hours as sufficient for such purposes. Whenever possible and in all cases of careful analytical work samples should be dried for three hours.

For this purpose observations as to the time required to reach constant weight were made on 23 samples of ice cream.

The dishes containing the ice cream were first weighed at the end of two hours drying in the oven and then again at intervals

TABLE 3

Percentage of solids and variation percentage of solids as obtained by the modified and Mojonnier method for determining total solids in ice cream

SAMPLE NUMBER	MOJONNIER METHOD	MODIFIED METHOD	MODIFIED FROM MOJONNIER	SAMPLE NUMBER	MOJONNIER METHOD	MODIFIED METHOD	MODIFIED FROM MOJONNIER
1	36.74	36.54	−0.20	26	38.74	38.43	−0.31
2	38.53	38.22	−0.29	27	37.41	37.29	−0.12
3	38.32	38.20	−0.12	28	38.22	38.10	−0.12
4	40.87	40.59	−0.28	29	41.49	41.25	−0.24
5	40.82	40.61	−0.21	30	37.39	36.95	−0.44
6	40.83	40.50	−0.33	31	41.45	41.25	−0.20
7	40.85	40.43	−0.32	32	38.12	37.94	−0.18
8	40.85	40.67	−0.18	33	38.66	38.62	−0.04
9	40.86	40.66	−0.20	34	35.73	38.55	−0.18
10	40.84	40.44	−0.40	35	39.76	39.61	−0.15
11	38.87	38.63	−0.24	36	38.70	38.40	−0.30
12	38.85	38.64	−0.21	37	39.07	38.86	−0.21
13	38.35	38.23	−0.12	38	42.50	42.41	−0.09
14	38.54	38.42	−0.12	39	38.62	38.34	−0.28
15	38.37	38.20	−0.17	40	37.28	37.00	−0.28
16	39.97	39.59	−0.38	41	35.82	35.60	−0.22
17	39.00	38.24	−0.66	42	35.43	35.17	−0.26
18	36.90	36.77	−0.13	43	40.62	40.47	−0.15
19	36.89	36.69	−0.20	44	38.80	38.42	−0.38
20	39.37	39.15	−0.22	45	38.19	38.00	−0.19
21	37.99	37.65	−0.34	46	35.37	35.14	−0.23
22	38.07	37.86	−0.21	47	36.61	36.52	−0.09
23	36.74	36.63	−0.11	48	38.86	38.63	−0.03
24	35.67	35.11	−0.56	49	38.64	38.49	−0.15
25	37.12	37.00	−0.12	50	39.12	39.10	−0.02
Average variation..........							0.223

of thirty minutes until constant weight was reached. Results are tabulated in table 4. If one considers samples that check within 0.05 per cent as having reached constant weight, all had reached constant weight at the end of three hours drying. However, 15 samples or 65 per cent had reached constant weight

It has been suggested by other workers that, as there is at present no offiical test for ice cream, as recognized by the Association of Official Agricultural Chemists, one of these methods should be proposed for acceptance as the official total solids tests for ice cream. Certainly in view of the increasing importance of the ice cream industry, there is need for a practical, accurate and reliable total solids test which can be considered as the official method. If such procedure seems desirable, the question arises as to which method should be recommended. For the sake of uniformity of methods, the adapted official test as suggested in this paper would seem well suited to meet the requirements, as it is based on a procedure similar to the official test for other dairy products. However, as already pointed out, this test while accurate and reliable, is laborious and time consuming, requiring from six to eight hours for completion. The modified method, as outlined in this paper, overcomes these objections. Basing conclusions on data secured, the authors believe the modified method is also accurate and reliable. Its simplicity and relative rapidity are features which tend to make it more practicable and acceptable than the adapted official method.

SUMMARY AND CONCLUSIONS

1. A total of 50 samples of ice cream was analyzed for total solids. Twelve of these samples were tested by three methods: (*a*) the Mojonnier, (*b*) a modified test similar to the one suggested for sweetened condensed milk, (*c*) a method adapted from the official method for determining solids in sweetened condensed milk. The other 38 samples were analyzed by the first two mentioned methods.

2. The Mojonnier method invariably gave higher results than the method adapted from the official test for condensed milk; the average of 12 samples was 0.483 per cent above.

3. The modified method in more than 80 per cent of the cases also gave somewhat higher results than the adopted official, but checked closer than the Mojonnier; in 12 samples the average difference was 0.226 per cent.

4. In comparing results on 50 samples analyzed by both the Mojonnier and the modified method, the Mojonnier invariably gave higher results. The average of these 50 samples was 0.223 per cent above the modified method.

5. Observations as to time required to reach constant weight in modified method seems to indicate that for all practical purposes such as determination of solids in commerical factories, two hours of drying in water oven is sufficient. Whenever time permits and in all cases of careful analytical work, samples should be dried for three hours.

6. The data as presented seems to warrant the conclusion that the modified method is a simple, economical and accurate method for determining the per cent of total solids in ice cream.

7. As at present there is no official method for determining the per cent of total solids in ice cream the two methods suggested (1) the modified and (2) the method as adapted from the official method for sweetened condensed milk should receive consideration, thought and study as to the desirability of having one of them recognized as the official method.

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THE USE OF TRUE AND IMITATION VANILLA EXTRACTS IN ICE CREAM[1]

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Every manufacturer of ice cream has, at some time or another, been confronted with the problem of determining the relative merits of artificial and true bean vanilla extracts. It has been claimed that the artificial extract will satisfy the same wants and desires as the geniune product. If the latter statement were to be accepted, arguments would not arise. However, the demand for the true bean extract is increasing rapidly enough to prove that it is still considered superior to the imitation, and the increased cost will not deter the buyer, if he seeks the best.

Because of the distinct taste which flavoring extracts impart to ice cream, it is important that they be of the best quality. The expense of the true extracts has been instrumental in encouraging the manufacture of many cheap imitations, as well as the production of extracts which have been diluted, or made from inferior beans, the flavor of which is often increased by the addition of vanillin and coumarin. The question naturally arises—is the manufacturer of ice cream getting what he is paying for when he buys a vanilla extract; also, how can he determine whether the extract is genuine or artificial.

The objects of this study were to determine the relative flavoring ability of true and artificial vanilla extracts in ice cream, and to determine the chemical and physical differences of the various extracts.

[1] This paper is a preliminary report, published as Contribution No. 15 of the Department of Agricultural Chemistry, The Pennsylvania State College.

TABLE 1

Testing experiments made on vanilla extracts

SAMPLE NUMBER	JUDGE 1			JUDGE 2			JUDGE 3			TYPE OF EXTRACTS DETERMINED BY ANALYSIS
	Kind	Strength	Quality	Kind	Strength	Quality	Kind	Strength	Quality	
10	True	Weak	Poor	Imitation	Fair	Fair	Imitation	Fair	Good	True
26	True	Fair	Good	True	Good	Excellent	True	Strong	Good	Imitation
27	Imitation	Strong	Fair	Imitation	Strong	Poor	Imitation	Strong	Good	True
28	Imitation	Fair	Good	True	Strong	Excellent	Imitation	Strong	Fair	Imitation
19	Imitation	Strong	Fair	Imitation	Strong	Poor	Imitation	Strong	Poor	Imitation
2	Imitation	Strong	Poor	Imitation	Strong	Very poor	True	Fair	Good	True
5	True	Fair	Good	Imitation	Fair	Poor	Imitation	Fair	Poor	True
23	True	Weak	Poor	True	Fair	Good	Imitation	Fair	Poor	True
24	True	Weak	Fair	True	Weak	Poor	True	Weak	Poor	Imitation
6	True	Weak	Fair	True	Strong	Good	True	Weak	Poor	True

EXPERIMENTAL

Twenty-five samples of extracts were obtained from various manufacturers. Ten samples were chosen at random—nothing being known about their composition. Chemical analyses were made on twenty-five samples, but as only ten of these were used in the experimental ice cream, results are given on only ten samples.

The first test was made by tasting the extracts to be examined—just as they were obtained from the manufacturer. Several men tasted the samples, which were in numbered bottles bearing no label, in an effort to classify the extracts as either true or

TABLE 2

Tasting experiments made on ice cream containing true and imitation vanilla extracts

SAMPLE NUMBER	TYPE OF EXTRACT BY ANALYSIS	NUMBER OF TIMES SELECTED BY JUDGES				
		First	Second	Third	Fourth	Fifth
10	True	3	2	3	2	0
26	Imitation	6	3	1	0	0
27	True	3	6	1	0	0
28	Imitation	1	2	6	1	0
19	Imitation	0	0	0	0	10
2	True	3	4	3	0	0
5	True	7	3	0	0	0
23	True	2	3	2	3	0
24	Imitation	2	2	5	1	0
6	True	2	2	6	0	0

artificial, and to judge the quality. After this initial test an endeavor was made to select the best "true" flavor and the best "imitation." The results are listed in table 1.

Ice cream mixes were prepared in which the vanilla extract was the only variable. After freezing each mix, five samples from each freezing were removed from the freezer and placed in the hardening room. An initial tasting test was made at the time of freezing, and a sample was removed from the hardening room every week for five weeks to determine the effect of storage on the persistence of the flavoring quality. The results of these tests are shown in table 2.

Chemical and physical examinations were made of the various extracts. The specific gravity, total solids, ash, percentage of alcohol and lead number were determined quantitatively. Qualitative tests were also made to determine the presence of coumarin and the methods ordinarily used to detect artificial extracts (1) were employed. The methods outlined by the Association of Official Agricultural Chemists (2) were used to obtain the specific gravity, total solids and ash, while the Wichmann method (3)

TABLE 3

Results of analysis on vanilla extracts

SAMPLE NUMBER	TYPE OF EXTRACT BY ANALYSIS	SPECIFIC GRAVITY 20°/4°	TOTAL SOLIDS	ASH	PER CENT ALCOHOL	LEAD NUMBER	COUMARIN
10	True	1.016	5.91	0.05	30.0	0.584	
26	Imitation	1.006	14.13	0.063	26.4	0.362	Present
27	True	1.060	34.39	0.24	24.0	0.785	
28	Imitation	1.066	30.60	0.04	26.0	0.226	Present
19	Imitation	1.034	23.84	0.001	26.4	0.284	Present
2	True	1.065	31.37	0.21	33.6	0.894	
5	True	0.999	27.51	0.07	42.0	0.996	
23	True	1.003	24.08	0.001	38.4	0.56	
24	Imitation	1.049	23.20	0.05	34.2	0.412	
6	True	1.140	57.50	0.20	0	0.916	

was used to obtain the lead number and the percentage of alcohol. Coumarin was also tested for by means of the Wichmann method (4).

Table 3 shows the analytical data obtained on the examination of the ten extracts used in ice cream.

DISCUSSION OF RESULTS

The initial test made by tasting the various extracts failed to enable the judges to classify an extract as genuine or artificial (see table 1). It should be remembered that these tests were not made by men who were vanilla experts. All of them, however, have had experience in the manufacture of ice cream, and should compare very favorably in ability with the average man in the plant who has to buy or select the flavoring extracts. An ex-

pert vanilla taster could undoubtedly tell the the difference between the various grades of vanilla by tasting, but the average man cannot.

When the samples of ice cream were judged for flavor, those containing the true flavors were selected the most frequently as being best. The samples to be judged were labelled with numbers, so that the judges did not know the nature of the extract used. One imitation, no. 26 (see table 2), compared very favorably with the true extracts. The flavor of the imitations did not persist as well as that of the true extracts during storage. It will be noticed that there was a considerable variation in the selection of the true extracts, no. 5 standing at the head of the list. Here, again, much difficulty would be experienced if we were to try to classify the extracts as true or imitation.

On examining the data obtained by analysis (see table 3), it will be observed that results obtained overlap each other—that is, the data on the imitation extracts is similar to that of the true extracts—with the exception of the lead numbers. This fact should be borne in mind when analyzing samples of vanilla. The lowest lead number of a true extract was found to be 0.56 and the highest 0.996. The lowest lead number for an imitation extract was found to be 0.226 and the highest 0.412. The percentage of alcohol in genuine extracts varied from 0 to 42 per cent, while in the artificial preparations the range was between 26 and 34 per cent. No analyzed samples fulfilled the requirements of the United States Pharmacopoeia. Sample No. 5, which had the highest lead number, also received highest rank in the tasting tests made on the ice cream samples.

SUMMARY

1. Tasting extracts will not enable the average ice cream manufacturer to determine the difference between a true and an artificial extract.

2. Tasting samples of ice cream will not enable the average ice cream manufacturer to tell whether or not a true or an artificial extract has been used in ice cream.

3. The experiments tend to show that true flavors persist better than the artificial.

4. The data obtained by chemical analysis can tell little with regard to the flavoring ability of the two general types of extracts.

5. If the Wichmann method is used to determine the lead number, an index may be obtained as to the genuineness of the vanilla extract. It should be remembered, however, that high lead numbers result from reinforcing extracts with excess vanillin, especially in the case of diluted extracts. The excess vanillin may be removed easily, however, thus nullifying its effect.

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